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Research Article

Characteristics of Umbilical Cord Derived Mesenchymal Stem Cell/UCMSC from *Macaca fascicularis* and Its Secretome Under Hypoxic Conditions

Alvian Dumingan[1], Amarila Malik[2*], Ratih Rinendyaputri[3, a], Hieronimus Adiyoga Nareswara Utama[1], Sunarno[3], Yoggi Ramadhani Purwaningtyas[3], Hasta Handayani Idrus[3], Rachmawati Noverina[4], Fathul Huda[5a], Ahmad Faried[6, a]**

[1]*Faculty of Pharmacy Universitas Indonesia, Depok 16424, West Java, Indonesia*
[2]*Division of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy Universitas Indonesia, Depok 16424, West Java, Indonesia*
[3]*Center for Biomedical Research, Research Organization for Health, National Research and Innovation Agency, Bogor, Indonesia 16911*
[4]*Bio Farma Stem Cell Research and Development, Bandung, Indonesia 40161*
[5]*Department of Neurology, Faculty of Medicine, Dr. Hasan Sadikin Central General Hospital/Universitas Padjadjaran, Bandung, Indonesia 40161*
[6]*Neurosurgery Department, Faculty of Medicine, Padjadjaran University, Bandung, Indonesia*
[a]*Graduate School of Biomedical, Sciences, Doctoral Program, Faculty of Medicine, Universitas Padjadjaran, Indonesia*

**Corresponding author: amarila.malik@ui.ac.id*

Abstract

Mesenchymal Stem Cell (MSC) secretome has potential as a neuroprotective and neuroregenerative agent. It can have effects due to its paracrine factors, such as Brain Derived Neurotrophic Factor (BDNF) and Stromal-Cell Derived Factor-1 (SDF-1) which can be induced with hypoxia preconditioning. This compound may play a role in the treatment of neurological diseases. Stroke has become a neurological disease that contributes to high rates of mortality and morbidity worldwide. There have been several pre-clinical trials on animal stroke models using MSC secretomes from rats and humans, but no studies have been conducted on Non-Human Primate, such as *Macaca fascicularis*. This species has been widely used in biomedical research and part of it can be utilized for such studies which will reduce the cost of using human MSC. The results of this study, Umbilical Cord (UC)-MSCs of *Macaca fascicularis* have been successfully cultured and characterized in terms of phenotypic and differentiation. Hypoxia precondition was able to induce BDNF secretion up to 264 pg/mL and SDF-1 up to 666 pg/mL in the UCMSC secretome. Hypoxic preconditioning with 3% oxygen can induce the most optimal BDNF and SDF-1 secretion, compared to 1% and 5% hypoxia.

Keywords: *BDNF; Hypoxia; Macaca fascicularis; SDF-1; UCMSC Secretome*

1. Introduction

Mesenchymal Stem Cell (MSC) is a stem cell that is widely used in therapy, which can secrete growth factors (such as Vascular Endothelial Growth Factor (VEGF) including Brain-Derived Neurotrophic Factor (BDNF)), Chemokines (such as Stromal-Derived Factor (SDF-1)/ CXCL12, and CCL2), and Cytokines (such as interleukin-6 (IL-6), IL-8, Tumor Necrosis Factor Alpha (TNF-α)) (Cunningham et al., 2018). When MSCs are exposed to hypoxia condition, they adapt to the microenvironment by reducing the appearance of oxidative stress, changing their metabolism to glycolysis, and increasing their motility to

tolerate hypoxia. This situation is also supported by the activation of the Hypoxia-Inducible Factor which can trigger direct and indirect pleiotropic effects (Pulido-Escribano et al., 2022). It makes growth factors, cytokines, and chemokines can be secreted 3-6 times more, thus increasing the function of proliferation, differentiation, migration, and inhibiting cell apoptosis (Cunningham et al., 2018).

The secretion of paracrine factors in the MSC culture medium is called the secretome. One particularly promising growth factor in secretome that functions as a neurotrophin is BDNF. This factor exerts its effects by promoting neuronal cell survival and differentiation through interaction with tyrosine kinase receptors (Liu et al., 2020). Another secreted protein that has potential as an adjuvant therapy is SDF-1. This protein has been shown to promote neuroregeneration after brain injury by stimulating the proliferation, differentiation, and migration of neural precursor cells. (Cheng et al., 2017). Many studies have been conducted to investigate the safety and therapeutic effects of the MSC secretome, particularly in diseases caused by central nervous system disorders such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis, Huntington's disease, ischemic stroke, spinal cord injury, and traumatic brain injury (Giovannelli et al., 2023; Pinho et al., 2020). Among the neurological diseases mentioned above, ischemic stroke stands out as it is the second leading killer disease worldwide (Johnson et al., 2019) and has become the number one cause of death in Indonesia (World Health Organization, 2024).

A stroke is a case of brain damage resulting from a non-traumatic circulatory disorder in the brain. This disease occurs suddenly, progressively, and rapidly due to thrombosis or embolism in the brain (Kementrian Kesehatan Republik Indonesia/Kemenkes RI, 2018)(Chance & Hickey, 1982). The treatment mainly aims to unblock the arteries and restore blood flow to the brain, which is usually given fibrinolytic agents to remove the thrombus, such as Tissue Plasminogen Activator (TPA) (Chester et al., 2019). In some patients, thrombolysis therapy is ineffective, necessitating the development of adjuvant therapy to enhance the efficacy of treatment. One such agent is neuroprotective and neuroregenerative, exemplified by MSC secretome (da Silva et al., 2023; Haupt et al., 2023).

The effects of MSC secretomes from both rats and humans have been seen through several pre-clinical trials on animal stroke models and prove MSC secretomes can improve nerve function, provide long-term neuroprotection effects, increase neurogenesis and angiogenesis, reduce the infarction zone in the brain, suppress nerve apoptosis, and improve motor recovery in animal models (Giovannelli et al., 2023). However, no studies have identified the types of growth factors, cytokines, and chemokines in MSC secretomes sourced from Non-Human Primates (NHP), such as the long-tailed monkey (*Macaca fascicularis*). These species are known to be organogenetically and genetically similar to humans but can minimize the ethical constraints encountered in using human samples (Mariya et al., 2019)(Krishnan et al.,

2022). Moreover, this species has been widely used in biomedical research and part of it can be utilized for such studies which will reduce the cost of using human UCMSC. Based on the above, this study aims to isolate and culture *M. fascicularis* UCMSC and determine the effect of hypoxic preconditioning (1%, 3%, and 5% O2) on cultured UCMSC under optimal conditions on the concentrations of BDNF and SDF-1 in the secretome.

2. Materials and Methods

Macaca fascicularis fetuses were collected from PT Biofarma, Indonesia, which has obtained ethical approval with document number: 224K-Mon-Nef01 by the Laboratory Animal Welfare and Use Commission of PT Biofarma. All UCMSC Isolation And Culture procedures were performed in a Biological Safety Cabinet (BSC)-*Thermo Scientific* aseptically, using pre-sterilized equipment (Rinendyaputri et al., 2023). The Umbilical Cord of *Macaca fascicularis* was taken at 120 days gestation through sectio caesarea. UC was cut with a thickness of about 2 mm and placed on 6-well plates, given a culture medium containing MEM, 10 - 20% FBS, and 1% PenStrep, and then cultured in incubator at 37 ^{o}C and 5% CO_2. The medium was changed every 2 days.

Phenotypic characterization was performed by flow cytometry using a kit from BD Bioscience and Flow cytometer (*BD Accuri™ C6 Plus*) to see positive and negative CD markers (Rinendyaputri et al., 2023). While characterizing the differentiation of UCMSCs using a kit from *StemPro-Gibco* to see the ability to differentiate into Adipocytes, Osteocytes, and Chondrocytes (Widowati et al., 2022). Observation of differentiation into Adipocytes by looking at lipid droplets using *Oil Red O*, Osteocyte differentiation looking at Calcium deposits using *Alizarin Red S* and Chondrocyte differentiation looking at Calcium deposits by *Alcian Blue*.

Hypoxia pre-conditioning was performed in a two-gas incubator-*Thermo Scientific*. UCMSCs that had reached 80% confluence were added to 10 mL culture medium without serum and then placed in the incubator. The preconditioning time was calculated since the oxygen concentration had reached 1%, 3%, or 5%, CO_2 concentration 5%, and the temperature had reached 37 ^{o}C. Hypoxia preconditioning was performed for 48 hours. Secretomes were obtained by centrifuging the culture medium at 1,200 rpm for 10 min, then the supernatant was taken and filtered using a 0.22 µm syringe filter (Rinendyaputri et al., 2023).

BDNF and SDF-1 measurements were performed according to the instructions of the Human BDNF ELISA Kit- Cusabio (Catalogue No.: CSB-E04501h) and Human SDF-1 Kit- ELK Biotechnology (Catalogue No.: ELK1183) (Rinendyaputri et al., 2023). After each step in the ELISA kit was followed, the solution in the well was read using an ELISA reader-*Bio-Rad* at a wavelength of 450 nm, then the concentration of BDNF and SDF-1 was determined. The data obtained were analyzed using *IBM SPSS Version 23.0*. To compare the results between several hypoxia-treated groups and the control, *One-way ANOVA* was used to compare results between multiple hypoxia-treated groups and against controls, followed by a *Post-Hoc Least Significant Difference* test.

3. Results and Discussion

3.1 Results

Macaca fascicularis umbilical cord explants were cut and placed in a 6-well plate in the center so that the MSC growth area when cultured becomes wider. UCMSC growth was observed using an inverted microscope at 100 times magnification. M. fascicularis UCMSC has a fibroblast-like morphology, predominantly characterized by a short spindle shape (Figure 1).

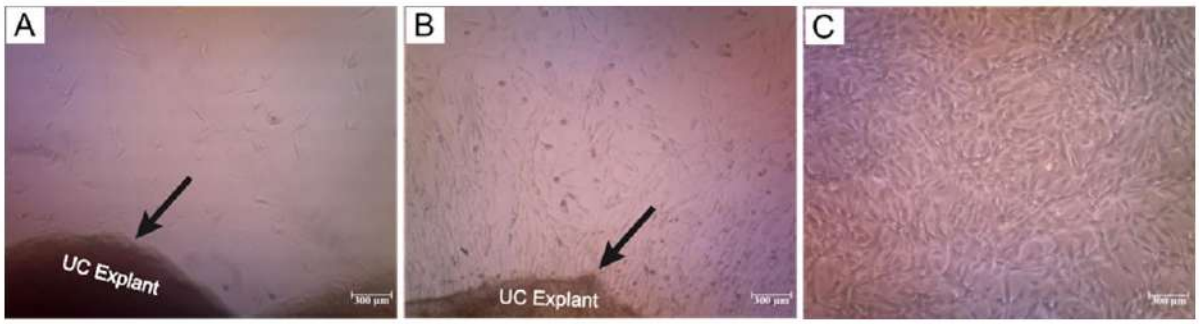

Figure 1. M. fascicularis Umbilical Cord (UC) Explant Day 9 (A). M. fascicularis UC Explant Day 14 (B). UCMSC after fifth passage (C)UC Explants are shown by arrows. After day 14, the growth of MSCs increased until MSCs filled the space of the well plate.

The characterization procedure for UCMSC was performed on cells from the 5^{th} passage. Positive results were seen for CD73, CD90, CD105, and CD44 as indicated by the presence of light scattering readings in the right-upper quadrant of the 5^{th} passage (Figure 2B). The negative CD marker cocktail reading confirmed the lack of CD34, CD11B, CD19, and CD45, as well as HLA-DR markers to differentiate MSCs from hematopoietic cell markers (Figure 2C). The characterized UCMSC can differentiate into the three cells indicated by being colored red for Adipocyte and Osteocyte Cells, while Chondrocyte Cells are colored blue (Figure 3).

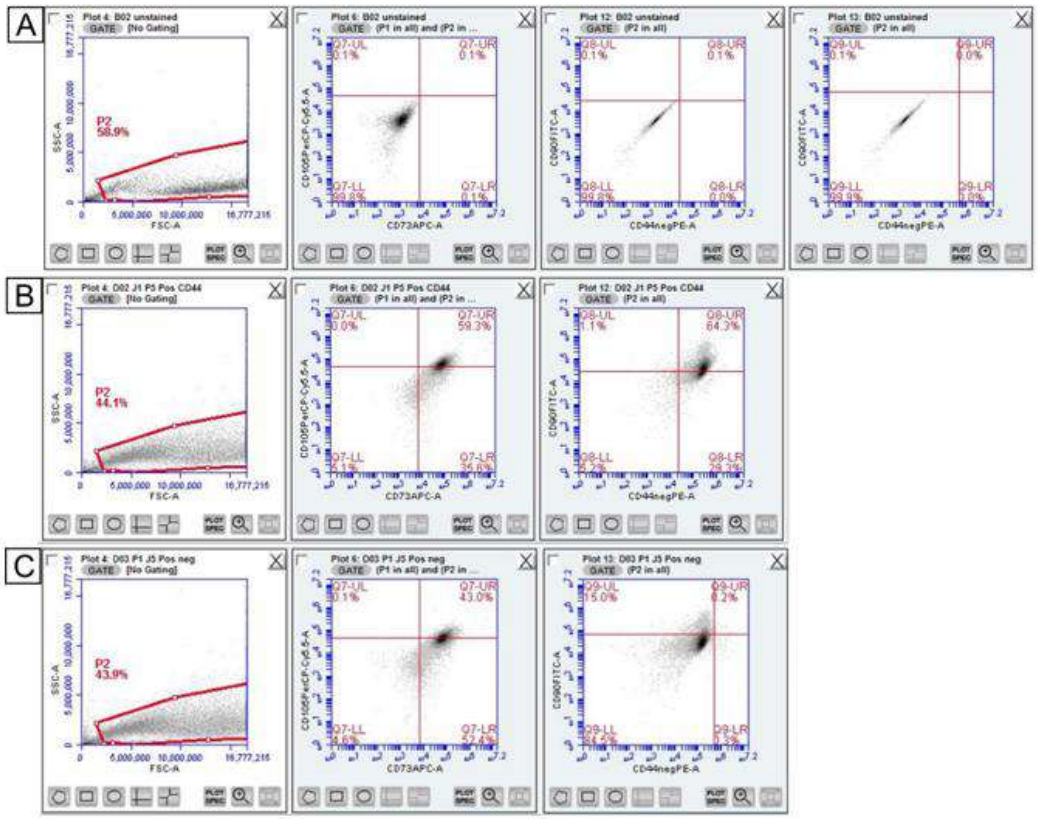

Figure 2. Reading of 5th passage unstained positive-negative marker cocktail (A). Reading of CD44 MSC Positive Marker 5th Passage (B). Reading of MSC Positive-Negative Marker 5th Passage (C).

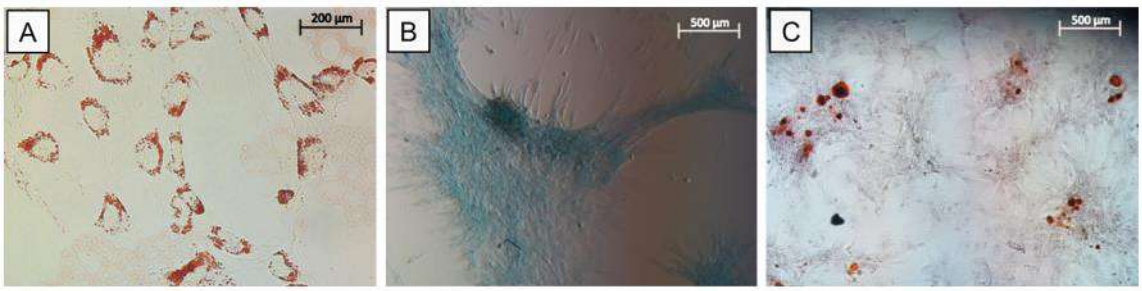

Figure 3. Results of UCMSC Differentiation Characterization. Differentiation of MSC Into Adipocytes (A), Chondrocytes (B), And Osteocytes (C).
Observation of adipocyte differentiation was performed at 100x microscope magnification, while chondrocytes and osteocytes were observed at 40x microscope magnification.

BDNF and SDF-1 profiles of UCMSC secretomes were examined using the ELISA method. Both standard curves used Exponential Association Regression, with the equation y=a(b-exp(-cx)). This equation has a correlation value (r) that is closest to 1.0000, so it is expected to provide a more accurate concentration calculation.

Based on the calculation results, hypoxia treatment was able to increase BDNF secretion up to 9 times and SDF-1 up to more than 100 times. BDNF and SDF-1 concentrations peaked in hypoxia conditions with 3% oxygen concentration. This

concentration was higher than when using a lower (1%) or higher (5%) oxygen concentration (Table 1).

Table 1. The concentration of BDNF and SDF-1 in Hypoxia-Preconditioned UCMSC Secretomes

Sample	BDNF		SDF-1	
	Concentration Mean (pg/mL)	*Std. Dev*	Concentration Mean (pg/mL)	*Std. Dev*
Kontrol	28.0	-	6.0	-
Hypoxia 1%	181.5	6.36	517.0	36.77
Hypoxia 3%	264.0	16.97	666.0	11.31
Hypoxia 5%	244.3	12.06	267.5	38.89

The results of statistical analysis showed that there were significant differences between the treatment groups, except for the mean BDNF concentration between the 3% and 5% hypoxia treatments. Likewise, in the mean SDF-1 concentration, non-significant differences were only found between the mean concentration of 1% and 3% hypoxia treatment (Figure 4).

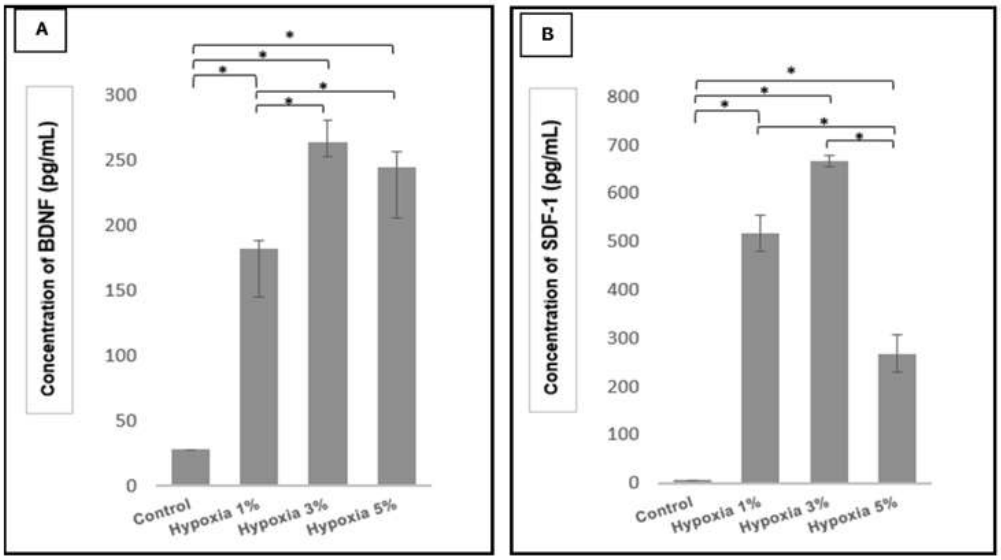

Figure 4. Concentration of BDNF (A) and SDF-1 (B) in *Macaca fascicularis* UCMSC Secretome with 1%, 3%, and 5% Hypoxia Preconditioning
* There is a significant difference between two treatment groups (P value <0.05)

3.2 Discussion

This study successfully obtained BDNF and SDF-1 at several concentrations in *M. fascicularis* UCMSC secretomes that had been cultured under optimum conditions with hypoxia preconditioning treatment (1%, 3%, and 5%). When associated with the cell culture stage, MSC sources from umbilical cord have better proliferation ability if compared to adipose tissue and bone marrow, and also show higher cell yield than sources from bone marrow (Mastrolia et al., 2019). The age of the MSC donor can also

affect the protein composition of the secretome (Turlo et al., 2023), thus using sources from fetal tissue can further limit the age variable. In this study, the umbilical cord was taken from *M. fascicularis* at 120 days gestation.

The culture medium used is Minimum Essential Medium (MEM) because it has been widely used to culture mammalian cells. This medium contains a large amount of amino acids so that it can mimic the protein composition in mammalian cells. In addition, this medium also contains glucose, vitamins, and minerals to provide nutrients to cells during culture. However, this medium does not contain protein, fat, and growth factors, so it is often combined with the addition of Fetal Bovine Serum (FBS) (Theodoridis & Kraemer, 2023). MSCs that will be used are verified against the minimum fulfillment of MSC criteria standards set by International Society for Cellular Therapy (ISCT), this is also important in data exchange to compare the results of this study with other similar studies (Dominici et al., 2006). The results of phenotype characterization and multilineage differentiation ability showed that the cultured cells met the minimum requirements (Fig. 2A-2C). Hypoxia conditions were applied to 5th passage UCMSCs in MEM medium without serum (FBS) because serum also contains other protein components that can interfere when analyzing data analysis.

Oxygen is one of the most important factors in metabolic processes and cellular signaling (Nakazawa et al., 2016). During cell culture, controlling oxygen levels is an important thing to consider. In general, cell culture is carried out in a state of normoxia, which is at a concentration of ~21% oxygen, which is higher than in vivo (Kaneko & Takamatsu, 2012). Oxygen levels in the atmosphere drop to around 14.5% - 19.7% after being inhaled through the mammalian respiratory system, then to 13.2% when passing through the arterial blood until it reaches the tissues, where oxygen levels remain around 0.7% - 7% (Carreau et al., 2011). The physiological oxygen concentration of MSC is 2-8% (Mohyeldin et al., 2010) and the oxygen concentration in umbilical cord blood ranges from 1-6% (Sjöstedt et al., 1960). Oxygen levels below atmospheric concentration (21%) during cell culture are often termed hypoxia.

The results of hypoxia preconditioning treatment at 1%, 3%, and 5% oxygen levels in this study showed the highest concentration of BDNF was at 3% hypoxia which was 264 pg/mL. This result is relatively higher when compared to similar studies that show the concentration of BDNF from human UCMSC secretomes in conditions of 10% hypoxia for 48 hours which is 50 pg/mL, and around 120 pg/mL after being treated with hypoxia for up to 96 hours with 5% oxygen concentration (Sidharta et al., 2018). Another similar study using MSC from gingiva, with hypoxia treatment for 7 days at 0.5 - 1% oxygen concentration was able to secrete BDNF in the amount of 150 pg/mL (Patil et al., 2022). MSCs from human umbilical cord Wharton's jelly can secrete BDNF around 4,500 pg/mL with hypoxia at 2% oxygen (Majumdar et al., 2013). This concentration is much more than the BDNF obtained in this study. The difference with this study is in the source of MSCs used, from Wharton's jelly, the use of *Knock out-DMEM* medium, and the method of

isolation by digestion instead of explants. When compared to the digestion technique, the explant technique has disadvantage in terms of contamination risk (Segeritz & Vallier, 2017).

Observation of SDF-1 concentration in this study also applies the same thing, where the highest concentration was obtained at 3% hypoxia, which amounted to 666 pg/mL. This amount is much more when compared to previous studies using MSCs from human Wharthon's Jelly tissue cultured in normal conditions without hypoxia preconditioning, which amounted to 2.0 pg/mL (Konala et al., 2020). There is also another study that used MSC source from human bone marrow and cultured in a transwell co-culture system with osteosarcoma cells grown in basal dishes, showing results slightly below this study, which was 600 pg/mL (Yu et al., 2015). SDF-1 in the secretome has also been obtained through 1% - 5% hypoxia preconditioning of human bone marrow MSCs that were concentrated 10 times using a protein concentrator and obtained an SDF-1 concentration of 850 ng/mL (Yang et al., 2023). The procedure of concentrating the secretome is a distinguishing factor that is thought to be the key to the high concentration of SDF-1 obtained compared to the current study.

Hypoxia conditions are known to affect the signaling of many cascades triggered by several transcription factors. Of the existing transcription factors, the Hypoxia Inducible Factor (HIF)-1α is a key factor that regulates cellular responses to hypoxia (Stamati et al., 2015). In the state of hypoxia, HIF-1α will form a heterodimer with HIF-1β which then binds to the Hypoxia-Response Element (HRE), which can affect the transcription of about 70 genes involved in various processes in cells, such as angiogenesis, metastasis, migration to cell death (Brahimi-Horn & Pouysségur, 2007). HIF-1α has the same signaling pathway as SDF-1, namely through the Phosphoinositide 3-Kinase (PI3K)/ Protein kinase B (Akt) pathway (Chen et al., 2013). The chemokine SDF-1 has several receptors to bind to, such as CXC Receptor (CXCR)-4 and CXCR-7 which play a role in cell migration. SDF-1 triggers PI3K/Akt signaling after binding to the CXCR-4 receptor (Haque et al., 2013). Activated Akt will trigger cell responses for proliferation, angiogenesis, migration, and survival (Chen et al., 2013). SDF-1 secreted in cerebral ischemic areas is also known to have a role in migration, proliferation, and prevention of cell apoptosis (Janowski, 2009).

The signaling pathway that occurs in BDNF, precisely mature-BDNF, also has a route that has a strong bond with the TrkB receptor. The phosphorylated TrkB will then activate several enzymes including PI3K and mitogen-activated protein kinase (MAPK) (Kowianski et al., 2917). In the PI3K/Akt signaling pathway, BDNF will modulate the synaptic plasticity of the N-methyl-D-aspartate receptor (NMDAR) which increases Ca2+ influx and is followed by the activation of cyclic-AMP response element binding protein (CREB) which ultimately triggers an increase in the expression of protein-coding genes that provide neuroprotective effects. While the MAPK / Extracellular-Signal-Regulated Kinase (ERK) signaling pathway can provide a protective effect on nerve cells from apoptosis (Zhao et al., 2017). BDNF works by minimizing cell death due to stroke,

increasing neurite/axon growth and neurogenesis after stroke, and inducing neural plasticity after stroke (Liu et al., 2020). The limitation of this study is that we could not determine the type of BDNF specifically whether it is pro or mature BDNF, we only got the total BDNF value.

Conclusion

M. fascicularis UCMSC has a fibroblast-like morphology, predominantly characterized by a short spindle shape. In addition, these MSCs have a positive phenotype for CD73, CD90, CD105, and CD44 markers and are capable of differentiating into adipocytes, osteocytes, and chondrocytes. Hypoxia preconditioning treatment can induce the secretion of growth factor (BDNF) and chemokine (SDF-1) from *Macaca fascicularis* UCMSCs, where 3% hypoxia can induce the most optimal compared to 1% and 5% hypoxia.

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Authors' Contribution

AD designed and performed testing as well as drafted the manuscript. AD, RR, AM, and SN validate the methodology and tests. AD, RR, and AM conduct the manuscript preparation. RR and AM supervised the entire study. All authors revised and approved the final manuscript.

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Research Article

Comparison of Antioxidant Activity of Methanol Extract of Young and Old Leaves of Avocado (*Persea americana* Mill.)

Ambar Pratiwi[1], Devanda Rahma Agustina[1], Novi Febrianti[2], Eri Eryati[3]

[1]*Department of Biology, Faculty of Applied Science and Technology, Ahmad Dahlan University.*
[2]*Department of Biology Education, FKIP, Ahmad Dahlan University.*
[3]*Biology Laboratory, Department of Biology Faculty of Applied Science and Technology, Ahmad Dahlan University.*

Corresponding author: ambar@bio.uad.ac.id

Abstract

Avocado plants (*Persea americana* Mill.) are widely grown in tropical and subtropical regions. Avocado leaves contain natural antioxidants for traditional medicine. This study aims to determine the total phenolic content, total flavonoids, and antioxidant activity values of young and old avocado leaves, and analyze the relationship between total phenol content and total flavonoids to the antioxidant activity value of avocado leaf methanol extract (*Persea americana* Mill.). The macerated avocado leaf extract was quantitatively tested for antioxidant activity using the DPPH method and the total phenolic and flavonoid contents were calculated using the Follin-Ciocalteu and Follin-Ciocalteu methods, respectively.The results showed that the highest total phenolic content of old avocado leaves was 1.682,27mg GAE/100g dry weight. The highest total flavonoid content of avocado old leaves was 3.858,96mg QE/100g dry weight. Antioxidant activity of methanol extract of young avocado leaves has a value of IC50 321.32 ± 80.43 ppm and avocado old leaves amounted to 288.54 ± 33.06 ppm. Total flavonoid content was more influential on antioxidant activity, with a correlation coefficient of 0.856.

Keywords: *Antioxidant Activity, extract, avocado leaves*

1. Introduction

Compounds that have the ability to prevent oxidation reactions are known as antioxidants. Antioxidant activity serves to counteract free radicals produced from the body, namely reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals, and peroxide radicals. Sources of free radicals can also be generated from external influences (exogenous) such as exposure to excess ultraviolet light, gamma radiation, environmental pollution, and cigarette smoke. High concentrations of free radicals will oxidize the molecules that make up cells, thus initiating the onset of diseases including cancer, hypertension, conditional disorders, and other degenerative diseases. Antioxidants work to stabilize compound reactions to prevent free radical damage (Anwar, 2014). Consumption of foods rich in antioxidant substances is one of the efforts in reducing radical exposure to the body. There are many sources of antioxidants in plants such as vegetables and fruits, including avocados. Apart from fruit, avocado leaves are also thought to have high antioxidant activity. Avocado plants (*Persea americana* Mill.), a member of the Lauraceae family, generally live in tropical to subtropical environments. Avocado production in Indonesia, according to data from the Directorate General of Horticulture and the Central Statistics Agency (2021), reached 669,260 tons. This number

has increased by 9.89% compared to last year, which was 609,049 tons. Central Java Province was recorded to produce 75,707 tons of avocado trees.

Avocado is a horticultural product with high economic value. Avocado pulp is used for consumption, while the seeds and leaves are used by the Indonesian people as alternative herbal medicines (Paramawati and Dumilah, 2016). Avocado leaves, which are seen as waste or organic waste, potentially contain antioxidants that can be used as traditional medicine such as antihypertension, rheumatism, antidiabetes, and kidney stones (Paramawati and Dumilah, 2016). The antioxidant ability of avocado leaves helps prevent oxidative stress related to various diseases. Avocado leaf extract produces flavonoid, saponin, tannin, and alkaloid compounds that can help prevent the onset of herpes simplex disease. Extraction with methanol solvent is able to extract compounds of both polar, semi-pattern, and non-polar characters. Methanol compounds can filter flavonoids, essential oils, saponins, tannins, and triterpenoids (Verdiana et al., 2018).

Widarta and Arnata's research (2017), shows the extraction process of avocado leaves with various solvents. The results showed that there were secondary metabolite compounds in avocado leaves including total phenols (23.28 mg/g) and total flavonoids (93.97 mg/g). The study also showed the antioxidant results of avocado leaves with the DPPH method (18703 mg/l). Phenolic is an organic material consisting of one or more bonds of hydroxyl substrate (OH). Phenol production is abundant in plants and is useful as a source of antioxidants (Puspitasari and Prayogo, 2017). Flavonoids are phenolic derivatives commonly found in leaves, fruits, stems, and flowers. Other antioxidant substances in leaves are flavonoids that can function to prevent cancer due to free radicals that can damage the cell structure (Waji and Andis, 2009).

One of the factors that affect the production of bioactive compounds produced in avocado leaves is the level of leaf age. Various studies show different flavonoid content in young leaves and old leaves of plants. Izzreen and Fadzelly (2013) reported that leaves (Camellia sinensis) have the lowest flavonoid compounds in old leaves compared to young leaves, while Mu'nisa et al. (2011), conveyed that old seagrass leaves produce higher flavonoids than young leaves. The results of another study stated that in tin leaves, the older the leaves, the lower the water content, tannins, and antioxidant activity of tin leaves and significantly affect the level of taste preference and overall acceptance (Amanto et al., 2020). The results of research by Felicia et al. (2016), explained that old avocado leaves with steaming techniques have a higher average value of total flavonoids than young avocado leaves.

The difference in the content of secondary metabolite compounds in young leaves and old leaves in plants is not yet known for certain. Thus it is necessary to conduct research to determine the comparison of total phenolic yield, total flavonoids, and antioxidant activity of avocado leaf extracts seen from the level of leaf aging.

2. Material and Method

2.1 Tools

The tools used in this research are analytical scales, blender, oven, 3 maceration jars, micropipette, measuring pipette 1 mL, 2 mL, 5 mL, and 10 mL, dropper pipette, 250 mL measuring cup, test tube and tube rack, 25 mL volumetric flask, 50 mL, and 100 mL, vortex, watch glass, spatula, stirrer, washing basket, knife, ruler, funnel, marker, waterbath, rotary evaporator, cuvette, UV-Vis spectrophotometer, incubator, and camera.

2.2 Materials

The materials used in this study are young avocado leaves with the criteria of light green color, smooth texture, taken 4-6 leaflets under the shoots, and old avocado leaves with the criteria of dark green color, smooth and firm leaf surface, taken 5-7 leaflets under young leaves originating from the Plikon area, Temanggung, distilled water, methanol, DPPH (1,1-diphenyl-2-picrylhydrazyl), Follin-Ciocalteu reagent, aluminum chloride reagent ($AlCl_3$), gallic acid, quercetin, ascorbic acid, Tris-HCl buffer (pH 7.5), Na_2CO_3, KCH_3COO 1M, aluminum foil, filter paper, tissue, and labels.

2.3 Methods

Young avocado leaves and old avocado leaves from the Plikon area, Temanggung were taken as much as 3000 g and 5000 g respectively. Samples of young avocado leaves and old avocado leaves were rinsed with running water and drained. The samples were placed in an oven for 24 hours at 40°C to dry (Widarta and Wiadnyani, 2019). The dried symplisia was pulverized into powder with a bleander, then sifted using a 60mesh sieve (Widarta and Wiadnyani, 2019). Young avocado leaf simplisia powder and old avocado leaves were taken as much as 100g and put in a dark maceration jar, added methanol solvent in a ratio of 1: 5 and covered with aluminum foil (Hayati et al., 2012). Then soaked for 2 x 24 hours at room temperature and protected from light, then the solution was filtered on filter paper with the help of a funnel to obtain filtrate (Talapessy et al., 2013). Avocado leaf extract is placed in a glass jar, then covered with aluminum foil, and can be tested for quantitative analysis.

The method to determine total phenolic content refers to the technique of Chun et al. (2003) in Malik and Ahmad (2015), with gallic acid for standard solution. The absorption wavelength was measured at 765 nm. Repetition5 was done 2 times (duplo) until the phenolic content obtained was obtained as mg gallic acid equivalent/g (mg GAE/g).

The method of determining total flavonoid content refers to the technique of Chang et al. (2002) in Ahmad (2014), with quercetin (QE) for the standard solution. Absorbance value was measured by UV-Vis spectrophotometry at a wavelength of 415 nm. The sample solution was repeated twice (duplo) until the flavonoid content was obtained as mg quersetin equivalent/g (mg QE/g)

Antioxidant Activity Testing DPPH method refers to the technique of Suyatmi et al. (2019), with ascorbic acid for standard solution. DPPH crystals in powder weighed 5 mg were dissolved in a 50 mL flask with methanol to obtain a 100ppm concentration DPPH solution. Sample concentrations of 0 ppm, 25 ppm, 50 ppm, 100 ppm, 200 ppm and 250 ppm and each made 2 repetitions (duplo). The absorbance value of the solution was measured using a visible spectrophotometer at an optimum wavelength of 517 nm, methanol was used as a blank, then the inhibition ability of DPPH radicals was calculated in percentages. Calculation of Inhibitory Concentration (IC50) to determine antioxidant activity was calculated by the equation formula (1).

$$\% \text{ Inhibition} = (\text{Abs. Control} - \text{Abs. Sample}) / (\text{Abs. Control}) \times 100\% \quad (1)$$

Data processing using normality test and analysis for correlation between total phenolic content, total flavonoids, and antioxidant activity of each methanol extract of avocado leaves using Pearson correlation. The results of the P value <0.05 state that there

is a significant relationship to determine whether or not there is a relationship between the total phenolic content and total flavonoids to the antioxidant activity of avocado leaf extracts.

3. Results and Discussion

3.1. Results

Avocado leaf simplisia is made by oven drying technique at 40°C. The temperature was chosen to avoid degradation of the structure of the content in the leaves such as flavonoid compounds with the nature of the substance easily oxidized and can't stand the heat if exposed to high temperatures. the highest average yield value is found in old avocado leaves of 22.5%, while young avocado leaves are 17.5%. This is because the greater the yield value indicates the value of the extract produced more and more. The requirement for the yield value of thick extracts is said to be good if the value is not less than 10% (Indonesia Herbal Pharmacopoeia, 2017). This means that the yield of young leaves and old leaves meets the requirements, because it is more than 10%. The use of methanol solvent for avocado leaf extract produces a high yield value, this indicates that methanol can extract compounds well in the leaves. Based on the nature of the solubility of a solvent can affect the acquisition of compounds in the extract (Badriyah and Farihah, 2022).

The total phenolic content of avocado leaf extract was carried out with gallic acid modification as a standard solution (Table 1).

Table 1. Test results of total phenolic content of methanol extracts of young and old leaves of avocado

Avocado leaf extract	Total phenolic content (mg GAE/100g dry weight)
Young leaves	1.454,55
Old leaves	1.682,27

The highest measurement for total phenolic content in old avocado leaves with a value of 1.682,27mg GAE/100g dry weight, while young avocado leaves have the lowest total phenolic content value of 1.454,55 mg GAE/100g dry weight. The above is in line with the research of Aziz and Jack (2015) on older *Nypa fruticans leaves that* have large phenol levels compared to young leaves. During plant growth, it is able to synthesize bioactive components and secondary metabolites in different amounts due to the influence of the level of leaf age and morphology (Farhoosh et al., 2007). Old leaf parts found trichomes as epidermal derivatives more than young leaves. This is because mature cell tissue (old leaves) has reached maximum growth, so that photosynthate is transferred to young leaves as a source of growth material. Biosynthesis of old leaves produces carbon atoms as a source of phenol secondary metabolites that function as a defense in plants from pests or other threats (Kuntorini et al., 2013).

Table 2. Test results of total flavonoids content of methanol extracts of young and old leaves of avocado

Avocado leaf extract	Total flavonoid content (mg QE/100g dry weight)
Young leaves	2.198,87
Old leaves	3.858,96

In Table 2. can be seen the content of the total flavonoid value of methanol extracts of young and old avocado leaves. Based on the data above, it is known that the highest total flavonoid compound measurement results are found in old avocado leaves with a value of 3.858,96 mg QE/100g dry weight, while young avocado leaves have the lowest total flavonoid content value of 2.198,87 mg QE/100g dry weight. The flavonoid content in this study shows that higher levels of compounds are found in old avocado leaves. The same thing was stated by Tehubijuluw et al. (2018), old seagrass tea leaves have a high flavonoid content of 0.1623%, while young seagrass tea leaves have a lower flavonoid content of 0.0888%. Factors that influence the yield of total flavonoids are the same as total phenols in that the increase in leaf age and morphology affects the content of bioactive compounds and the production of secondary metabolites (Farhoosh et al., 2007). Old leaves have better maturity of constituent components, because they contain sufficient nutrients and chlorophyll as a catcher of sunlight, resulting in a high rate of photosynthesis. The high rate of photosynthesis goes hand in hand with the production of secondary metabolites, one of which is flavonoids, which are formed in old leaves more than young leaves. Carbon compounds from photosynthesis are useful as a source of secondary metabolite formation (Tehubijuluw et al., 2018).

Antioxidant activity test research was conducted using the DPPH method which is a stable radical compound. The use of DPPH to measure the activity of antioxidant substances is based on their ability to capture free radicals. According to Suyatmi et al. (2019), the advantages of the DPPH method are a simple, fast, simple and sensitive way of analysis for low concentration samples, but testing with DPPH is limited because DPPH can only dissolve in organic solvents, making it difficult to analyze hydrophilic compounds.

Increasing concentration of the sample solution, the percentage of inhibition against DPPH is getting higher. The percentage of inhibitory activity or inhibition of young leaves and old avocado leaves increases mutually. At a concentration of 250 ppm, the highest inhibition of old avocado leaves was 45,98%, while young avocado leaves were 44,90%. The higher the concentration, the higher the percentage of inhibition, so that the reaction of the sample with DPPH is stronger and more stable. This is because more DPPH binds to hydrogen particles in the extract tested to reduce the absorption of DPPH. The results of this measurement are relevant to Molyneux (2004), that the percent inhibition of DPPH can affect the determination of antioxidant activity, if the inhibition of a sample is high, the higher the activity of antioxidant substances in the sample. The antioxidant activity test was conducted using the DPPH method to determine the activity level of the sample in inhibiting the stable DPPH radical through hydrogen atom donation. Samples with antioxidant activity reduce DPPH to DPPH-H (Molyneux, 2004).

Table 3. Measurement IC50 antioxidant activity of methanol extract of young and old leaves of avocado

Avocado leaf extract	Values IC50 (ppm)
Young leaves	321,32±80,43
Old leaves	288,54±33,06

Table 3. shows the value of IC50 antioxidant activity of methanol extract of young avocado leaves amounted to 321.32 ± 80.43ppm and old avocado leaves amounted to 288.54 ± 33.06ppm. Based on the calculation of the value of IC50 antioxidant activity shows that the sample of methanol extract of old avocado leaves has higher antioxidant activity than young avocado leaves. The antioxidant activity of old avocado leaves is categorized as very weak because the value is more than 200 ppm. IC50 more than 200 ppm, but still has potential as an antioxidant compound (Tristantini et al., 2016). The extract sample produced a weak antioxidant presumably due to the presence of impure compounds that have not been separated from some other component substances, so it is necessary to carry out a fractionation process for the separation of golonga. value IC50 is used as a good determination of the antioxidant efficiency of pure compounds or extracts. According to Molyneux (2004), the lower the value, the higher the antioxidant activity of the compound. IC50 the higher the antioxidant activity of the compound. The result of the value of IC50 The value of old avocado leaves shows smaller than young leaves, meaning that the antioxidant content is greater in old avocado leaves. This is due to the level of leaf age can affect the production of secondary metabolites in a plant. The increasing age of the leaves, the levels of bioactive compounds that act as antioxidants such as flavonoids are increasing.

Antioxidant properties in avocado leaves can be influenced by the biosynthesis mechanism of secondary metabolites such as flavonoids or phenol compounds. These compounds include types of chemical compound components of antioxidant substances that are abundant in plants. According to research by Achakzai et al. (2009) in Felicia (2016), phenolic and flavonoid content compounds are higher in old leaves than young leaves. The age of the old leaves, the antioxidant activity is high, because the concentration of phenols and flavonoids including secondary metabolites that act as antioxidants is higher.

Pearson correlation test based on the results of the table above shows that the total flavonoid content has a p-value <0.01, while for the total phenolic content has a p-value> 0.01 can be seen from the large correlation coefficient. The relationship between the total flavonoid content of avocado leaf methanol extract and DPPH free radical inhibition has a positive correlation with a coefficient value of 0.856. This result states that the total flavonoid content has a strong relationship with the antioxidant activity of a sample.

Conclusion

The conclusion of this study is that the highest total phenolic content of avocado leaf methanol extract (*Persea americana* Mill.) is found in old leaves amounting to 1.682,27mgGAE/100g dry weight. The highest total flavonoid content of avocado leaf methanol extract (*Persea americana* Mill.) was found in old leaves at 3.858,96mgQE/100g

dry weight. The highest antioxidant activity value of avocado leaf methanol extract (*Persea americana* Mill.) is found in old leaves amounting to 288.54 ± 33.06 ppm. The total flavonoid content in the methanol extract of avocado leaves (*Persea americana* Mill.) has more influence on antioxidant activity, with a correlation coefficient of 0.856.

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Research Article

Identification of Laboratory Management Systems at Senior High Schools In Mataram City

Eni Suyantri, Baiq Sri Handayani, Tri Ayu Lestari, Heru Setiawan

Departement of Biology Education, Faculty of Teacher Training and Education, University of Mataram, Mataram

Corresponding author: enisuyantri@unram.ac.id

Abstract

The Laboratory for Biology serves an effective educational facility for learning of biology, where biology subject often requires lab activity. Proper management of the laboratory is a crucial aspect for the smooth execution of practical activites by both teachers and students. The objective of this research was to determine the biology laboratory management system at SMA Negeri 3 Mataram. This research is descriptive research using observation and interview methods. The data collected in two types, the primary data is observation questionnaire and the result of interview to the biology teachers of SMAN 3 Mataram. The development of the observation questionnaire for the standardization of biology laboratory refers to the National Education Minister's Regulation No. 24 of 2007, and the laboratory management standards refer to The Minister of Education and Culture's Regulation No.8 of 2018. There are ten aspects considered in the observation of the biology laboratory, including the evaluation of the laboratory space, the completeness of laboratory equipment and materials, and the cleanliness of the laboratory. Based on the research found that eight aspects were categorized as very good, and two aspect were categorized as as good. The completeness of laboratory tools and materials, as well as laboratory safety, need to be improved.

Keywords: *laboratory management system, biology laboratory, standardised laboratory.*

1. Introduction

The laboratory is a place where practical activities, experiments, measurements, scientific research (chemistry, physics, biology) or other scientific research are carried out. Science laboratory act as academic component in educational institutions, play an important role to support teaching and support the success of the skills learning process. Science laboratory with the lab activities provide a better explanation for students, biology teachers should prepare teaching materials followed by practical activities or experimental methods.

Science laboratories as an educational infrastructure, need to be maintained the effectiveness of it function. To maintain the effectiveness of its functions, a science laboratory must be managed and organized well with clear operational mechanism. Laboratory management is a technique or method for achieving objectives through the stages of planning, organizing, directing, and supervising all laboratory potentials to achieve optimal good targets (Ali, 2018).

Laboratory management is crucial for the effectiveness and efficiency of laboratory's operation (Elseria, 2016). A laboratory, as a place of learning, would be better

managed before it is used. Proper management facilitates the use of the science laboratory for both teachers and students. Planning, organizing, implemention, supervision, and evaluation are essential components for the successful management of a laboratory.

Laboratories are used in education to train intellectual skills through observation, recording natural phenomena, and developing students' motor skills (Silvester *et al*., 2023). This activity teaches students to use the tools they have to seek and find the truth, on it nurtures the courage to persue scientific truth the natural and social environment. It also teaches them to be careful, patient, honest, think critically and skillfully.

According to The Minister of National Education No. 24 of 2007 concerning Facilities and Infrastructure Standards for Senior High School General Education, every senior high school is required to have at least one designated room for a biology laboratory. School laboratories play a crucial role in biology education, as certain learning materials necessitate observation and experimentation. If the school laboratory fails to meet these standards, practical activities will be hinderd, resulting unachieved learning objectives.

The biology laboratory serves both educational and research purposes, implementing and advancing biological theories and concepts. The practical activities conducted in this laboratory are intended to enhance students' comprehension of various forms of knowledge, including facts, concepts, principles, laws, and theories. Effective biology education relies on practical, hands-on learning experiences. Consequently, biology practical activities require adequate support in terms of facilities and infrastructure, such as laboratories and their associated equipment. This underscores the significance of biology laboratories within the school biology curriculum. Mulyasa (2006), biology laboratories constitute a vital resource for learning.

Considering the significance of laboratory laboratory as an educational resource, therefore very crucial to assess the facilities of laboratory at SMA 3 Mataram. The assessment should include the laboratory's layout and capacity of the laboratory equipment, tools and materials for practical activities, safety apparatus, and other laboratory resource at SMAN 3 Mataram.

2. Material and Method

The research methodology employed was descriptive research. The object of this research was SMAN 3 Mataram, with the participants involved in this research activity were biology teachers who in charge as the laboratory assistants. The data sources used were divided into two, primary data and secondary data. The primary data for this study were derived from questionnaires and observations of biology laboratory standardisation. The operation of the laboratory were conducted according to the Regulation of The Minister of National Education No. 24 of 2007 and standardisation of laboratory management according to the regulation of The Minister of Education and

Culture No. 8 of 2018. Secondary data were obtain from the interviews with biology teachers about the subject matter taught during biology practical activities.

The data analysis technique employed in this research is inductive data analysis. Inductive data analysis is a process whereby conclusion are drawn from specific facts, and then generalization are made. The steps involving in analysis data in this study are data collection, data reduction, data display and data retrieval.

The questionnaire data, expressed as assessment points, converted into percentage and subjected to analysis. The formula used to measure the percentage is as follows:

$$expected\ score = \frac{total\ score}{maximum\ score}\ x\ 100\%$$

The formula was used to analyse the results of the questionnaire that has been distributed to teachers and biology laboratory assistants. The criteria for evaluating the aspects of biology laboratory management are as follows.

Table 1. Criteria for assessing aspects of biology laboratory management

Percentage	Category
86 % – 100%	Very good
76 % – 85 %	Good
66 % – 75 %	Good enough
55 % – 65 %	Not good enough
0 - 54 %	Very bad

3. Results and Discussion

3.1. Results

Based on the questionnaire used to collect research data, there were ten aspects of assessment compiled based on biology laboratory standardization referring to Regulation of The Minister of National Education No. 24 of 2007 and standardized laboratory management referring to regulation of The Minister of Education and Culture No. 8 of 2018. The ten aspects in detail as follows.

Location and Laboratory Space

The biology laboratory room at SMAN3 Mataram is 10 m x 15 m and divided into three rooms. The first of these is a storage room or warehouse measuring 4 m x 5 m, which is used as a storage area for tools and materials, The second is a preparation room measuring 4 m x 5 m, which is used for preparation of practical activities. The third is a practical activity room measuring 10 m x 11 m, which is used for practical activities or teaching and learning activities. In accordance with the finding of Kertiasa (2006), the optimal laboratory configuration for high school level comprises three distinc areas:

student practice rooms, preparation rooms and storage rooms. This was also stated by Rustaman et al. (2003), who asserted that the laboratory encompasses a primary area (practice room) and additional space (preparation room and storage room). Based on these observations, the biology laboratory room at SMAN 3 Mataram has found to aligned with the standard. Ismiyanti et al. (2021) stated that the layout of the laboratory is important because it is related to the comfort and safety of students engaged in practical activity activities.

A biology laboratory that meets the standards of The Minister of National Education No. 24 of 2007 is a laboratory that has a practical activity/student work space with a student movement ratio of 2.4 m^2/student. The average number of students in one study group of SMAN 3 Mataram who use the biology laboratory for practical activity is 36 students. Referring to The Minister of National Education No. 24 of 2007, with an area of 150 m^2, the ratio obtained is 4.1 m^2/student. The laboratory of SMAN3 Mataram is quite spacious and able to accommodate a minimum of one study group for each practical activity. The size of the laboratory is quite large, so it is not an obstacle or barrier for students to carry out practical activity. The laboratory also has a clean water source, but the number of clean water taps is minimal. In addition, all laboratory rooms also have good and adequate lighting facilities. Good lighting will make it easier for students and teachers to carry out practical activity.

Completeness of Laboratory Tools and Materials

The completeness of laboratory tools and materials at SMAN3 Mataram was adequate but there were some tools and materials that are not found in the laboratory. From 39 lists of tools and materials listed in the questionnaire, there were 9 tools and materials that were not found in the laboratory of SMAN3 Mataram. Among them are wire mesh, meiosis preparations, mitosis preparations, pictures of plants from various divisions and phylum, pictures of human breathing, pictures of human blood circulation, pictures of human excretion, and pictures of human reproduction. Most of the laboratory tools and materials can be found in the preparation room and some others are found in the laboratory warehouse. Tools found in the biology laboratory of SMAN3 Mataram in accordance with the standards of The Minister of National Education No. 24 of 2007 including Petri dishes, beakers, object glasses, cover glasses, funnels, measuring pipettes, test tubes, three legs, Bunsen, veterinary surgical instruments, balance sheet, Erlenmeyer, human body skeleton model, human body odle, DNA images, RNA images, preparation boxes, digestion images, human circulatory system images, monocular microscopes, binocular microscopes, plant anatomy preparations, animal anatomy preparations, sterilizers. While the materials found are spiritus liquid, alcohol, KOH, $NaHCO_3$, $CuSO_4*H_2O$, $CuSO_4$ 1%, glucose and $CaCO_3$. There are several tools and materials that can be found but do not meet the standards, including measuring cups, test tube clamps, stopwatches and e-tubes.

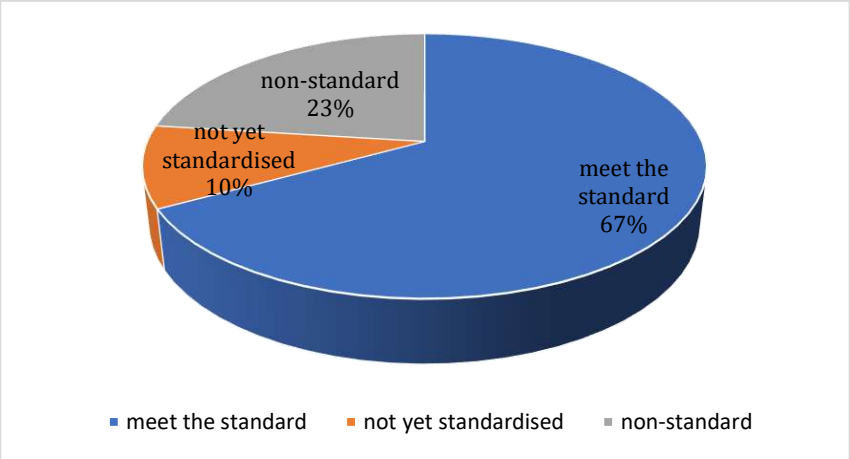

Figure 1. Comparison of the completeness of tools and materials that have met the standard.

Storage of Laboratory Equipment and Materials

The storage of laboratory equipment and materials in the biology laboratory of SMAN 3 Mataram is conducted appropriately. Equipments and materials are stored separately in separate cabinets. Upon removal of tools and materials from the storage cabinet, the glass cover of the cabinet must be closed again to maintain the sterilization of the existing tools and materials. The storage cabinets for tools and materials are sufficiently robust and secure for storage of laboratory equpments and materials. The storage cabinets are locked to prevent the loss of laboratory equipment and materials. The available storage cabinets were numerous and of a considerable size, allowing for the accommodation of a multitude of practical activity tools and materaials. The separation of storage for tools is commandable, with the majority of tools being stored in the preparation room and damaged tools being stored in the laboratory warehouse. However, the cabinets are not labeled with the names of the tools stored, making it challenging to locate the necessary tools. The result of the research, revealed that one item on the questionnaire did not align with the conditions observed in the laboratory of SMAN 3 Mataram. In general, the storage of tools and materials was conducted appropriately. however, there are still some areas that require further attention.

Laboratory Equipment

Biology laboratory equipment at SMAN3 Mataram was complete, proven by the 23 points of the questionnaire have been fulfilled entirely. The equipment available in the biology laboratory of SMAN3 Mataram as follows: blackboard, electrical outlet, wall clock, strong and stable demonstration table, sink, clean water source, strong chair for student practical activity, teacher or laboratory chair, and strong and stable practical activity table.

Maintenance of Laboratory Equipment

The maintenance of biology laboratory equipment at SMAN 3 Mataram was done well. Materials and equipments were always reorganized after each practical activity. The feasibility of the equipment was monitored by the laboratory manager. Laboratory equipment was cleaned after each use so that it can be used for the next practical activity. There were periodic reports on equipment damage recorded by the laboratory manager. Some laboratory equipment will be repaired if it can still be repaired while still paying attention to the efficient use of laboratory equipment. Tools used in practical activity were always recorded before doing practical activity by students. Periodic checking or maintenance of laboratory equipment is always carried out by the manager or laboratory assistant.

Laboratory Organization and Administration

In order for the continuity of the laboratory's usefulness to be maintained, the laboratory needs to be managed properly. One part of this laboratory manager is the staff or personal laboratory. The laboratory staff or personnel were responsible for the effectiveness and efficiency of the laboratory including facilities, equipment and materials for practical activity.

SMAN3 Mataram has a laboratory organizational structure consisting of a laboratory head, laboratory technicians and laboratory personnel. The head of the laboratory is authorized and responsible for planning, implementing, developing, evaluating and following up all activities related to biology laboratory activities. Laboratory technicians are authorized and technically responsible for the preparation of practical activity, storage, maintenance and care of biology laboratory equipment and materials. Laboratory staff are administrative staff who are responsible for managing and carrying out laboratory functions, laboratory administration, and controlling laboratory usage.

In the administrative aspect, there is a laboratory management plan, a laboratory management plan made by the laboratory assistant, and a standard operating procedure (POS) for laboratory work. In addition, there is a schedule of laboratory activities and a report on laboratory activities. The duties of the technicians are detailed and formulated by the laboratory assistants. There is also a work schedule for technicians and laboratory assistants adjusted to the class hours at the school. The need for laboratory materials, equipment and spare parts is well planned. After conducting practical activities, there are periodic practical activity reports done by students. And once every six months (one semester), program evaluation activities are carried out to improve laboratory activities. During practical activities, there is an attendance book that needs to be filled in by teachers and practicioners while in the laboratory room. Furthermore, there is also a record of the use and damage of equipment in a book carried out by the laboratory assistant. All practical activities are periodically written in a report book.

Laboratory Utilization

Laboratory utilization at SMAN3 Mataram is carried out systematically equipped with a practical activity guidebook, and there are 6 practical activity courses that are carried out. Grade 11 conducted practical activity on animal and plant anatomy, human excretion and human digestion. Grade 12 conducted a practical activity on photosynthesis. The use of the laboratory is adjusted to the existing laboratory use schedule. The laboratory at SMAN 3 Mataram is not shared with other schools or institutions. The laboratory is used for research or discovery of innovations by students and teachers. There are several scientific papers resulting from studies or innovations. In addition, there are some catalogs available for research planning. To improve students' knowledge and understanding, practical activities are always carried out to support the material in biology subjects.

Provision and Preparation of Tools and Materials to be Used for Practical activity

In the biology laboratory of SMAN3 Mataram, there is a preparation table that is strong, stable, safe, and the size of the table is sufficient to prepare the tools and materials for practical activity. Before the practical activity is held, the laboratory manager or laboratory assistant identifies the tools and materials according to the needs of the practical activity to determine the availability of tools and materials in the laboratory. The provision of tools is done through checking the tools to find out which tools are still functioning properly or not. The laboratory assistant prepares instructions for using laboratory equipment to avoid damage to the tool when used and to get accurate results from using the tool correctly. Laboratory assistants prepare tools, materials, guides, and supporting equipment for practical activities to facilitate practical activities carried out by students. The laboratory assistant acts as a helper for the preparation of practical activity tools and materials, periodic checking, maintenance and storage. Thus, laboratory assistants are needed in the laboratory (Indriastuti, 2013).

Laboratory Safety

Laboratory work safety is the best condition in conducting practical activity which is equipped with equipment to conduct experiments or investigations. Laboratory work safety needs to be prioritized to prevent work accidents. To support work safety, a laboratory must be equipped with equipment that supports the safety of laboratory assistants and practitioners or students. Work safety in the biology laboratory of SMAN3 Mataram is quite good. The laboratory is equipped with fire extinguishers that can be operated when accidents occur during the practical activity process, such as fires and explosions. There is a first aid kit in the preparation room, but there is no wound medicine. There is no checking of the expiration date of the drugs in the first aid kit, as evidenced by one of the drugs that has expired. First aid equipment tends to be incomplete. In the implementation of practical activity, hazardous and toxic materials

always apply their own procedures and are always monitored in handling. Each individual is always required to maintain personal health and safety, as well as the work environment. Overall, the condition and safety of the laboratory building is in good condition.

Cleanliness of Laboratory Room and Furniture

The cleanliness of the biology laboratory room of SMAN3 Mataram needs to be considered in order to support practical activities carried out by teachers and students. The laboratory of SMAN3 Mataram has been equipped with trash bins, sinks, tables and chairs in a clean and tidy condition. Laboratory equipment is neatly organized in the preparation room. However, some equipment and materials in the warehouse are not clean and neat. Even so, the waste from the practical activity can be resolved properly. Cleaning checks before and after practical activity are always carried out to maintain the sterilization of equipment and materials in the laboratory.

The following are the results of the percentage assessment of each aspect in accordance with The Minister of National Education No. 24 of 2007 biology laboratory SMAN3 Mataram.

Table 2. Results of identification of biology laboratory management system at SMAN3 Mataram

Aspects of the assessment	Results	Category
Location and Laboratory Space	90%	Very good
Completeness of Laboratory Tools and Materials	77%	Good
Storage of Laboratory Equipment and Materials	91%	Very good
Laboratory Equipment	100%	Very good
Maintenance of Laboratory Equipment	100%	Very good
Laboratory Organization and Administration	100%	Very good
Laboratory Utilization	87,5%	Very good
Provision and Preparation of Tools and Materials to be Used for Practical activity	100%	Very good
Laboratory Safety	75%	Good
Cleanliness of Laboratory Room and Furniture	87,5%	Very good

3.2. Discussion

The finding of the research and analysis revealed that eight out of ten aspects of the assessment of the biology laboratory at SMAN 3 Mataram were in the very good category, two aspects of the assessment yielded results in the good category, as presented in Table 2. These results indicate that the management system of the biology laboratory at SMAN 3 Mataram has been in accordance with the standardization set out in Permendiknas No. 24 of 2007. This is supported by the completeness of the biology laboratory layout at SMAN 3 Mataram which has a good laboratory layout. Based on these

results, it can be said that the management of laboratory management is done well. Awell managed laboratory is one that has effective organizational system, clear job descriptions, and good spatial planning in building planning. These elements are carried out effectively and efficiently with a disciplined work system (Suranto et al., 2020).

The availability of laboratory equipment and materials is an important factor in the implementation of practicum activities in schools. Candra and Hidayati's research (2020) indicated that the limited equipment for practical activities will impede the implementation and process skills, as well as work skills of students. The biology laboratory of SMAN 3 Mataram requires the following tools and materials: wire mesh, meiosis preparations, mitosis preparations, images of plants from various divisions and phyla, images of human respiration, images of human blood circulation, images of human excretion, and images of human reproduction. Additionally, the laboratory requires an increased in number of measuring cups, test tube clamps, stopwatches, and ethanol. The procurement of tools and materials for practical activities must be based on needs (Ambarwati and Prodjosantoso, 2018). The laboratory plays an essential role in developing skills and fostering lifelong learning, as well as increasing student interest and enabling students to engage continuous learning. Therefore, it is crucial to ensure the completeness of laboratory equipment including clothing, with gloves and footwear, to ensure the safety of students and the efficacy of laboratory activities (Atunuva and Artun, 2020).

Storage space is a fundamental component of any laboratory infrastructure. The findings indicated that the storage room in the biology laboratory of SMAN 3 Mataram was effectively managed and compartmentalized according to the designated storage areas for tools and materials. Suranto et al. (2020) asserted that the storage space for equipment, particularly those crafted from metal, must be separated from chemicals to prevent damage to tools or contamination of materials. The findings also demonstrate that biology teachers at SMAN 3 Mataram, in their capacity as laboratory managers, possess high competence in laboratory management. Singerin (2022) in his research mentioned that one of the signs of laboratory managers' competence is their ability to organize the storage of tools, materials and other spare equipment in school laboratories.

Adequate learning laboratories must be supported by effecttive laboratory equipment maintenance. It is recommended that equipment maintenance be carried out on a scheduled basis and equipped with a maintenance control card to facilitate monitoring of laboratory equipment (Suranto et al., 2020). The findings of the study indicated that the maintenance of laboratory equipment at SMAN 3 Mataram was conducted in an exemplary manner by the laboratory manager, who undertook regular inspections and promptly addressed any damage equipment. Wang (2017) stated that one of the characteristics of good laboratory management is the presence of good maintenance management, including the repair of damaged laboratory equipment and the ability to devise solutions to any challenges that may rise

The utilization of the builogy laboratory at SMAN 3 Mataram was 87.5% with the very good category. Indicators of laboratory use include the conduct of various practical activities such as animal and plant anatomy, excretory and digestive systems. This utilization is supported by other parameters such as room availability, excellent equipment availability, and excellent equipment maintenance. Indirasari et al. (2019) stated that there is a positive correlation between the availability of laboratory facilities and the motivation and achievement of learning objectives. Laboratory utilization in biology learning provide students with direct experience in science learning and increases their understanding of scientific phenomena (Etiubon and Ufonabasi, 2020).

Laboratory work safety is a crucial aspect that must be given due to consideration, as it is directly related to the performance of laboratory assistants, teachers, and students. The greater the availability of work safety and security facilities, the lower the likelihood of work related accidents. First aid kits and fire extinguishers are essential tools that must be readily accessible in the laboratory (Rahmantiyoko, 2019). However, the drugs found in the biology laboratory of SMAN3 Mataram were not checked for expiration, and there were no burns or external injuries. It is evident that laboratory safety is a shared responsibility of both managers and users. It is imperative that everyone involved in laboratory activities possess a sufficient level of awareness regarding laboratoey safety management. Teachers and students in particular, are among the users of laboratory facilities who require a comprehensive understanding of laboratory safety management (Adilah et al., 2021) In addition to the aforementioned considerations, the management of laboratory safety also encompasses the availablility of pertinent work safety regulations, including those pertaining to the in handling of materials and equipment, which serve to protect students engagement in laboratory activities (Akinbobola and Olufunminiyi, 2015).

Conclusion

Laboratories are very important in schools to carry out practical activities. The ideal laboratory criteria standards are listed in The Minister of National Education No. 24 of 2007 and The Minister of Education and Culture No. 8 Of 2018. Based on the results of the assessment of the ten aspects of the assessment, eight of them are in the very good category and two of them are in the good category. Two aspects that need to be improved are the completeness of tools and materials and work safety in the laboratory. Based on the identification of all aspects, it can be said that the system is very good.

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Review Article

The Effect of Using E-modules in Biology Learning on Learning Outcomes of High School and University Students: Meta-Analysis

Nurfadila Arifin[1], M. Harja Effendi[2], Nining Purwati[3]

Department of Biology Education, Universitas Islam Negeri Mataram, West Nusa Tenggara, Indonesia

Corresponding author: harjaefendi@uinmatarm.ac.id

Abstract

This study aims to determine the effect size (ES) of the application of e-modules in biology learning on student's learning outcomes. This study uses the type of meta-analysis research. The research sample consisted of 14 articles sourced from the results of article identification through *Google Scholar* and *Eric.* The research data were collected based on the reference of inclusion and exclusion criteria. Data analysis was conducted by calculating the ES value using *Cohen's d* formula. The results of data analysis showed that there were 14 articles divided into two levels of education, namely high school (9 articles) and university (5 articles) with a total average effect size score of 1,14 which means the effect size criteria are very large (VL). The ES average score for the high school level is 1,20 or very large (VL) ES average, while the ES average score for the university level is 1,02 or categorized as large (L) ES average. Therefore, it can be concluded that the effect size of the application of e-modules in biology learning has a significant impact on improving student learning outcomes.

Keywords: *E-modules, Biology, Student Learning Outcomes, Effect Size*

1. Introduction

Learning outcomes are abilities or competencies that involve three aspects, namely cognitive, affective, and psychomotor abilities. Learning outcomes are also used as a reference to assess how far students are in understanding the material presented (Imansari & Sunaryantiningsih, 2017). High and low student learning outcomes can be influenced by internal and external factors. Internal factors that affect student learning outcomes are physiological factors. Physiological factors describe a person's mental and physical condition. This factor is very important in helping students to remain in a stable condition in learning. External factors that affect student learning outcomes include parental environment, teacher competence, teacher communication, learning motivation, learning methods, and learning media (Jamil, 2017),

Learning media is one of the most important parts that support the success of a lesson. Learning materials can be delivered well and will be easier to understand with the help of learning media. Learning media will help students learn either independently or in groups which can improve learning outcomes and build students' critical thinking skills (Alfariez & Nadiah, 2023). In the current era of globalization, learning media is required to be more creative, innovative, and effective by developing technology-based learning media that can be used in an effort to improve student learning outcomes (Citra & Rosy, 2020).

Of the various technology-based learning media that are one of the learning supporters in the world of education that are able to attract special attention because of their potential to change the current traditional education paradigm are electronic modules or e-modules. E-modules are technology-based textbooks designed by teaching staff or teachers with the aim that students can learn independently under the guidance of the teacher in a systematic manner (Hunaidah et al., 2022). E-modules that not only contain text, but are supported by video, audio, and pictorial animations make e-modules more interesting and not boring. E-modules offer various features such as ease of navigation and users are facilitated by the display of images, video, audio, animation, and e-modules also allow immediate feedback (Rahmi et al., 2021).

Biology is often perceived as a subject that contains a lot of memorized text, abstract, and scientific words which then make students difficult and reduce their interest in learning (Maryanti & Kurniawan, 2018). The presence of e-modules with more supporting features can make learning biology more interesting and can help teachers visualize biological material which is often considered abstract. So e-modules are expected to be an interactive learning media that can improve concept understanding and student learning outcomes (Dewi & Lestari, 2020).

Some previous studies that tested the effectiveness of the use or application of e-modules in learning biology to improve student learning outcomes both at the high school and college levels showed positive results in the relationship between biology e-modules and student learning outcomes. Research conducted by Wulandari et al (2020) shows the results of increasing motivation and learning outcomes of grade X students in biology learning. In addition, development research as well as experiments to develop sigil software e-modules also show results that can improve student learning outcomes (Munandar et al., 2021). Both studies are also supported by research conducted by Rodiyah et al (2023) related to the development and influence of e-modules on reproductive system material in class XI students which shows the effectiveness of e-modules in improving student learning outcomes. Based on these studies, proves that the e-module media has a great influence in improving students' biology learning outcomes. Therefore, researchers are interested in testing the Effect Size of each experimental research related to the development and application of e-modules in learning biology on student learning outcomes at the high school and university levels.

2. Material and Method

This research uses the Meta-Analysis research model. Meta-analysis is research that explains a quantitative approach and is designed to review the results of previous research (Hartati, 2022). To achieve the research objectives, this study is based on two research questions as follows: 1) Whether the application of e-modules in learning biology in high schools and universities have a significant effect on student learning

outcomes?; 2) What is the percentage of the effect size of the application of e-modules in learning biology on the ability of student learning outcomes?

Research data searches were conducted using "Google Scholar" and "Eric" with the keywords "E-module" OR "Biology" OR "Student Learning Outcomes" OR "Paired t-test". The research data in the form of articles obtained through Google Scholar and Eric searches are selected based on the inclusion and exclusion criteria described in the table as follows:

Table 1. Inclusion and Exclusion Criteria

Criteria	Description
Inclusion	1. Articles with a publication year of the last 5 years (2020-2024) 2. SINTA-indexed journals (S1-S4) for Indonesian journals 3. Articles that use experimental or quasi-experimental research methods 4. Display paired t-test data 5. Articles with the scope of high school and university biology
Exclusion	1. Articles with publication year below 2020 2. Not indexed by SINTA or Scopus 3. Display half or none of the paired t-test data.

Data collection was carried out using the PRISMA method. PRISMA (Preferred Reporting items for Systematic Reviews and Meta-Analysis) has systematic stages, namely formulating (Identification), searching for literature systematically (screening), filtering or selecting articles deemed appropriate to the researcher's title (eligibility) and analyzing articles that have been selected (included) (Zarate et al., 2022). The articles that were successfully identified through the PRISMA method were then given an "F" code on each article. The following is the PRISMA diagram of the research data filtering process flow:

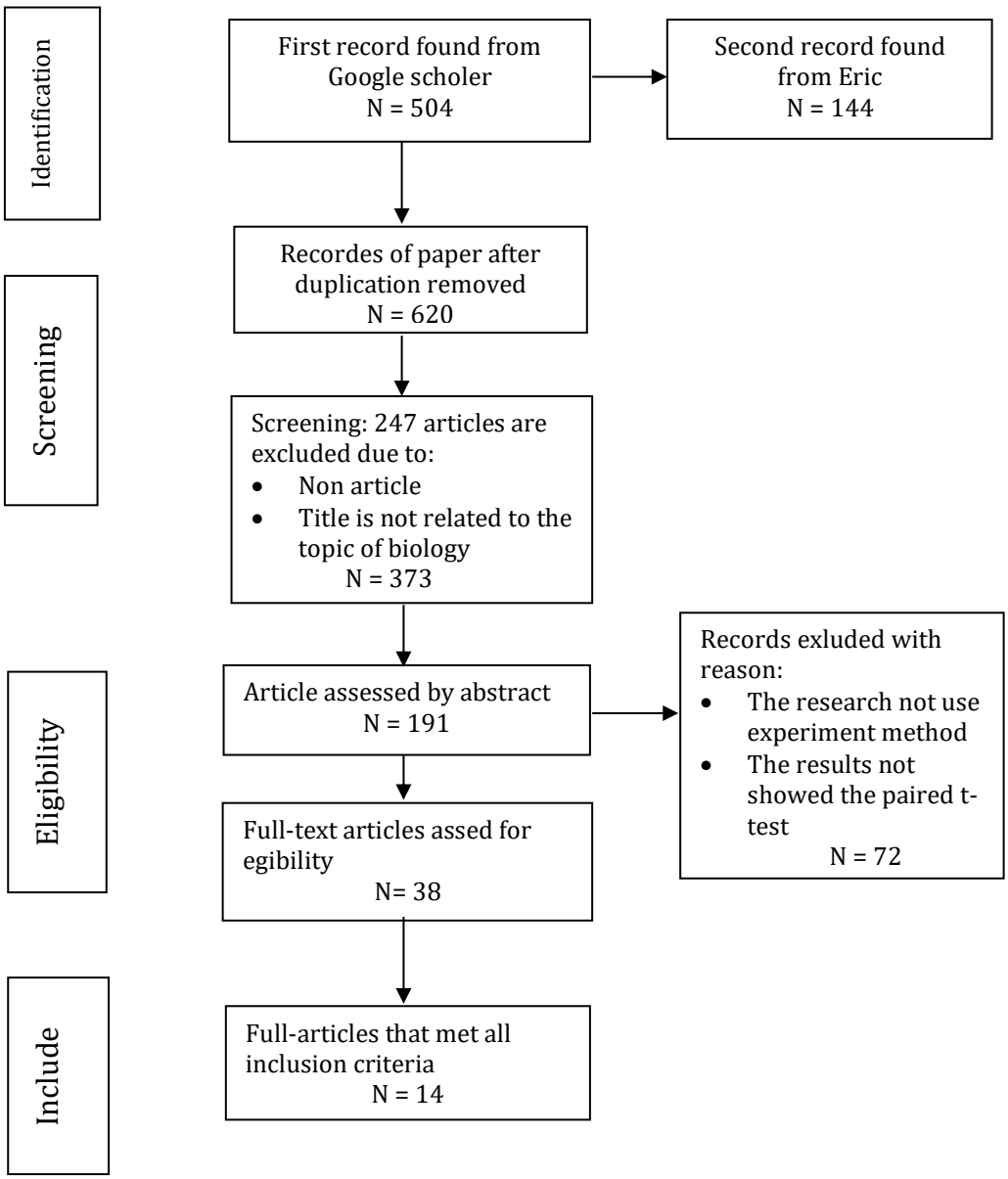

Figure 1. PRISMA diagram of research data collection

Analysis of research data using paired t-test data from each article using the cohen's d formula to determine the effect size of the application of e-modules on student learning outcomes in biology. The calculation of effect size uses Conhe's d formula which involves the difference in mean and standard deviation of the t-test results (Samo et al., 2023). The following Cohen's d formula was used to calculate the effect size of the 14 articles:

$$d = \frac{M_{2-}M_1}{SD_{polled}}$$

Description:
d : the size of the effect size
M_1 : average of group 1 (control class)
M_2 : average of group 2 (experimental class)
SD_{polled}: combined standard deviation

The combined standard deviation value of the two groups was obtained using the following formula:

$$SD_{polled} = \sqrt{\frac{S_1^2 + S_2^2}{2}},$$

Description:
S_1^2 : control group variance
S_2^2 : experiment group variance

Furthermore, to determine the criteria of ES, the calculation results through Cohen's d formula are determined by following the following references:

Table 2. Effect Size Criteria

ES	Criteria
ES ≤ 0,15	Very small (VS)
0,15 < ES ≤ 0,40	Small (S)
0,40 < ES ≤ 0,75	Medium (M)
0,75 < ES ≤ 1,10	Large (L)
ES > 1,10	Very large (VL)

Source: Mayasari et al., (2022)

3. Results and Discussion

3.1. Results

Based on the results of searching and identifying articles through Google Scholar and Eric, 14 articles were obtained related to the effect of biology e-modules on the learning outcomes of high school and university students. The data from the effect size analysis of 14 articles are described in the following table:

Table 3. Effect size analysis in general

No	Code	Author, year	Level	Effect size (ES)	Criteria
1	F1	(Subhan, 2022)	High school	0,97	L
2	F2	(Lasala, 2023)	High school	1,25	VL
3	F3	(Delita et al., 2022)	Uni	0,96	L
4	F4	(Supratman et al., 2023)	High school	2,4	VL
5	F5	(Darmawulan et al., 2022)	Uni	0,40	S
6	F6	(Mulyaningsih & Mubarok, 2022)	High school	0,72	M
7	F7	(Maghfiroh et al., 2023)	High school	1,2	VL
8	F8	(Jimenez, 2020)	High school	0,57	M
9	F9	(Cruz & Mariana, 2023)	High school	1	L
10	F10	(Sumarmi et al., 2021)	Uni	2	VL
11	F11	(Sagita, 2021)	High school	1,9	VL
12	F12	(Masing & Aminatun, 2022)	High school	0,85	L
13	F13	(Sudarmika et al., 2024)	Uni	0,16	S
14	F14	(Koth et al., 2021)	Uni	1,58	VL
Total average effect size				**1,14**	**VL**

Based on the effect size analysis table above, the effect of e-modules in biology learning on student learning outcomes shows the highest value of 2.4 and is categorized in the criteria of very large (VL) influence or means that there is a very high influence between the application or use of e-modules in biology learning on student learning outcomes. The lowest effect size value is 0.16, which means that the effect of e-modules on student learning outcomes is small (S).

Table 3 Analysis of average effect size based on education level

Education level	Code	ES Average	Criteria
High school	F1	1,20	Very large (VL)
	F2		
	F4		
	F6		
	F7		
	F8		
	F9		
	F11		
	F12		
University	F3	1,02	Large (L)
	F5		
	F10		
	F13		
	F14		

Table 3 shows that the average value of ES at the high school education level is categorized as very large (VL) with an average effect size of 1,20. At the University level, the average value of ES is 1.02 and is categorized as large (L).

3.2.Discussion

Research related to the development of technology-based learning media is a topic that has been chosen by many researchers. One type of technology-based learning media that is in great demand by researchers is e-modules. Based on search data through PRISMA with the theme of biology e-modules, the number of research results reached 648 articles of various levels in the last five years. This proves that this research is currently the topic of choice among researchers in the field of education.

The application of e-modules in biology learning can affect student learning outcomes. Learning outcomes are the result of interpreting the relationship between a learning interaction and a teaching interaction (Delita et al., 2022). Learning outcomes also illustrate student success in taking a course of study. According to Bloom's taxonomy, learning outcomes can be divided into three, namely cognitive knowledge (understanding of concepts), affective (attitudes), and psychomotor (process skills) (Andriani & Rasto, 2019). Several researchers who researched to test the effectiveness of an e-module in learning biology on student learning outcomes showed mixed results.

Based on data analysis of the effect of e-modules in biology learning on student learning outcomes that have been tabulated in general table 2 shows a high average ES value between the two variables, namely the use of biology e-modules and student learning outcomes. Effect size is a natural representation of “how wrong” the null hypothesis is in quantitative terms or a measure between treatment and outcome (Hedges, 2008). ES analysis is carried out to determine the standardized difference

between the values or scores of the control and experimental groups. The ES score of a study can be done with several existing formulas, one of which is Cohen's d.

A total of 14 articles have been tested for effect size using cohen's d formula and show the highest ES result of 2.4 and is categorized as very large criteria (VL). Based on table 1 ES criteria, ES results are categorized as very large (VL) if the ES score is more than 1.10. There are 10 articles that show a large effect size score (4 articles) and very large (6 articles) which means that research on the use of e-modules in biology learning has a very significant positive effect on improving student learning outcomes, both at the high school and university levels. This is in line with Jeniffer's opinion that the application of technology-based learning media can increase student motivation and engagement, thereby also increasing student academic achievement (Harris et al., 2016).

Then, there are 2 articles that fall into the medium (M) criteria category with ES scores of 0.75 and 0.57. The calculation results with Cohen's d formula also show that there are 2 articles that have a small (S) ES score ($0.15 < ES \leq 0.40$), namely 0.40 and 0.16. The small ES score indicates that the effect of e-modules in biology learning does not significantly improve student learning outcomes. However, the comparison of the number of articles that state a significant positive effect size of e-modules in learning biology on student learning outcomes is more than the articles that state medium or small effect size values.

This research focuses on biology topics at the high school and college or university levels. The results of the research data identification show that the use of e-modules in biology learning is more widely applied in high schools. Based on Table 3 analysis of the average ES based on the level of education, the results show that at the high school level, there are 9 articles collected with an average ES of 1.20 with very large (VL) criteria category. Meanwhile, at the university level, there were 5 articles with an average ES score of 1.02 with a large (L) criteria category. The average value of ES from both levels of education related to the effect of e-modules in biology learning on student learning outcomes proves that the effective use of technology-based learning media has succeeded in improving student learning outcomes at both levels.

The success of e-modules in improving student learning outcomes in biology learning is inseparable from the interactive nature of e-modules. E-modules are designed to display several features such as animated images, videos, audio, and text that can help visualize the contents of the module and make readers easily understand difficult material (Ricu Sidiq & Najuah, 2020). In addition, e-modules are also able to strengthen students' interest in learning independently and improve the quality of learning (Dewi & Lestari, 2020). Thus there is a positive reciprocal relationship between the use of e-modules and student learning outcomes. The results of the effect size analysis of 14 articles through Cohen's d formula prove that the use of e-modules in learning biology can significantly improve student learning outcomes.

Conclusion

Based on the Effect size analysis by calculating the mean and standard deviation values of 14 articles using cohen's d formula, the following conclusions can be drawn: 1). The application of e-modules in biology learning has a significant effect in improving student learning outcomes. 2). The ES score for the high school level is 1.20 and categorized in very large (VL) criteria, while the ES score for college or university level is 1.02 or categorized in large (L) criteria. Of the 14 articles collected, 2 articles had ES scores with small (S) criteria. However, overall the average effect size shows a positive influence of the application of e-modules in biology learning and increased student learning outcomes.

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Research Article

Bioactivity Diversity (Antibacterial and Antioxidant) of Macroalgae in the Ekas Beach Area, Lombok

Baiq Repika Nurul Furqan[1], Imam Syahputra Yamin[2], Sri Sofiati Umami[3]

[1]*Departement of Pharmacy, Universitas Nahdlatul Ulama Nusa Tenggara Barat*
[2]*Departement of Nutrition, Universitas Nahdlatul Ulama Nusa Tenggara Barat*
[3]*Departement of Biology Education, Universitas Islam Negeri Mataram*

Corresponding author: repika.nf@gmail.com

Abstract

Macroalgae in the southern coastal area of Lombok has not been explored much in terms of bioactivity. The aim of this research is to explore the usefulness of 9 types of macroalgae on Ekas Lombok beach by searching for their bioactivity capabilities, whether each macroalgae has no bioactivity at all, has antibacterial and antioxidant activity, only has antibacterial activity but no antioxidant activity or vice versa and whether there are macroalgae that even have anticancer activity. The method used in this research consists of several steps: Macroalgae Sampling, Morphological Analysis of Macroalgae Samples, Isolation of active compounds from macroalgae and Cytotoxic Test on Breast Cancer Cells in Vitro which consists of 3 tests: Culture and sub culture of MCF-7 Breast Cancer Cells, Cell Viability and Cytotoxicity Test with MTT and DNA Isolation of MCF-7 Breast Cancer Cells. The research results show that the macroalgae with the best antibacterial activity is the red macroalgae *Acanthophora spicifera* and the macroalgae with the best antioxidant activity is the green macroalgae *Ulva reticulata.*

Keywords: *Bioactivity diversity, antiocsidant, antibacterial, macroalgae, Ekas beach*.

1. Introduction

Indonesia is a maritime countries that has a very diverse range of macroalgae (seaweed). This diversity is not accompanied by the use of seaweed in Indonesia, which is still far behind when compared to other seaweed producing countries such as Japan, Korea, Taiwan and China. The use of seaweed production in Indonesia is still limited to the food sector and commodity raw materials for export. Meanwhile, in other countries, seaweed has been widely used as an ingredient in industries other than food, such as the pharmaceutical, cosmetic, medical and agricultural industries (Liu et al, 2019).

One of the islands in Indonesia that has abundant macroalgae diversity is the island of Lombok, both the east, west, north, central and even the south coast of Lombok have a variety of macroalgae. 9 types of macroalgae have been successfully identified morphologically and genetically, 4 types of red macroalgae (*Acanthophora spicifera, Eucheuma sp., Gracilaria foliifera* and *Wurdemannia miniata*), 3 types of brown macroalgae (*Hormophysa cuneiformis, Padina australis* and *Turbinaria ornata*) and 2 types of green macroalgae (*Ulva intestinalis* and *Ulva reticulata*) (Furqan et al, 2023).

Macroalgae identified from other areas such as beaches in West Java (most data has been successfully explored at Sayang Heulang Beach) have been studied for their

bioactive compound content and have been successfully tested for their bioactivity as antioxidants, antibacterials, anti-inflammatory and even anticancer. Therefore, researchers are interested in conducting research related to the diversity of bioactivity of 9 types of macroalgae on Ekas beach, Jerowaru (South Coast of Lombok).

The aim of this research is to explore the usefulness of 9 types of macroalgae on Ekas Lombok beach by searching for their bioactivity capabilities, whether each macroalgae has no bioactivity at all, has antibacterial and antioxidant activity, only has antibacterial activity but no antioxidant activity or vice versa and whether there are macroalgae that even have anticancer activity.

2. Materials and Methods

2.1 Materials

The tools used in the study were clear plastic, cooler box, micro pipettes measuring 1–10 L, 10–100 L, and 100–1000 L (Biologix), mortar and pestle, tweezers, 100 mL measuring cup (Iwaki), Erlenmeyer 100 mL (Iwaki). The instruments used consisted of a DSC-H300 digital camera (Sony), MAC-601 autoclave (EYELA), micro centrifuge (Prism TM R), TM water bath (MyBath), vortex mixer (Labnet), refrigerator (Toshiba), analytical balance (Ohaus Pioneer), PCR machine (BioRad), agarose gel electrophoresis apparatus (Biorad), GPS (Global Positioning System) (Garmin), thermometer, multiparameter HI 9828 (Hanna Instruments), light microscope (Nikon) and UV wave transilluminator 254 nm (Vilber Lourmat).

The seaweeds used in this study were 4 types of red macroalgae (*Acanthophora spicifera, Eucheuma sp., Gracilaria foliifera* and *Wurdemannia miniata*), 3 types of brown macroalgae (*Hormophysa cuneiformis, Padina australis* and *Turbinaria ornata*) and 2 types of green macroalgae (*Ulva intestinalis* and *Ulva reticulata*) taken from Ekas beach, Lombok. Breast cancer cells (MCF-7) were obtained from the cell culture laboratory, Faculty of Medicine, Mataram University Teaching Hospital. Solvents used for extraction and column fractionation were n-Hexane (p.a, Merck), methanol (p.a, Merck), acetone (p.a, Merck), chloroform (p.a, Merck), 0.05 M phosphate buffer solution (pH 7, 6) which is made from KH2PO4 and K2HPO4 solutions.

The materials used to test antibacterial activity were Escherichia coli, Staphylococcus aureus and P. acne (bacteria obtained from the Mataram Health Laboratory), Tryptone, yeast extract, NaCl, Agar and Tetracycline and kanamycin as positive controls. The ingredients used to test antioxidant activity were DPPH (1,1-diphenyl-2-picryhydrazyl) (Sigma) and Gallic Acid (Sigma) as positive controls.

The materials used for cell culture and cell harvest were RPMI (Gibco), FBS 10% (Gibco), Penicillin-Streptomycin (Gibco), PBS (Invitrogen), DMSO (Merck) and EDTA (Merck). For the cytotoxicity test, MTT (3-(4-5-dimethylthiazol-2-yl-)-2,5-diphenyltetrazolium bromide) (Sigma) and Doxorubicin (Dankos) were used as positive controls.

Cancer cell protein isolation using the Nuclear Extraction Kit (Abcam ®), DNA Methyltransferase Activity Test using the DNMT1 Inhibitor Screening Assay Kit (Abcam ®).

2.2 Methods

Macroalgae Sampling

Sampling of macroalgae was carried out in tidal beach conditions with sunny weather. Seawater conditions at the time of sampling were analyzed with several parameters, including: pH, temperature (°C), conductivity (µS/cm), total dissolved solids (ppt) and salinity (PSU). Seawater analysis was carried out on each square that was submerged in seawater during sampling.

Morphological Analysis of Macroalgae Samples

Morphological analysis was carried out based on visual observations and documented using digital camera photos. Parameters observed included color, shape and branching of the thallus. The morphology of macroalgae samples was evaluated and compared with the morphology of macroalgae found in the literature (Guiry and Guiry, 2018).

Isolation of active compounds from macroalgae

Samples of 9 types of macroalgae, each of which had been dried and finely ground, were then extracted using the method from Kumari et al., (2018) with several modifications. The dried samples were macerated for 24 hours at room temperature using methanol (p.a), chloroform (p.a) and 50 mM phosphate buffer pH 7.6 with a ratio of 2 : 1 : 0.8 (v/v). Then filtered to get the first filtrate. The remaining residue was re-extracted 3 times using the same solvent in a ratio of 1 : 1 : 0.8 (v/v) for 30 minutes, filtered and a second filtrate was obtained. The extraction was repeated until the fourth filtrate was obtained. The collected filtrate was then added to Milli-Q in a ratio of 9 : 2 and centrifuged for 15 minutes at 2100 xg. The results of centrifugation will separate the mixture into 2 phases, namely the chloroform phase and the methanol-water phase. The two phases are separated and each phase is concentrated using an evaporator. The chloroform phase obtained was then weighed, while the methanol-water phase obtained was dried using a freeze dryer to obtain dry powder from the crude methanol-water extract and weighed.

Fractionation of the crude extract of each methanol-water was carried out using the column chromatography method according to the method of Behbahani, et al (2013) with several modifications, namely by using column chromatography, G60 7733 silica with a height of 10 cm and a diameter of 1.2 cm. Elution was carried out in stages using a mixture of hexane and acetone with a ratio of 8:2, 5:5, 2:8 (v/v) respectively and ended with methanol (p.a). The total volume of each eluent is 24 mL for the mixture of hexane and acetone, and 50 mL for the methanol eluent. The silica gel to be used is activated by

heating at 100°C and equilibrated with hexane: acetone in a ratio of 8: 2 (eluent 1) overnight to prevent the silica from breaking. The crude extract (0.1 – 0.5 grams) was added with 5 drops of methanol then put into the column. The elution flow rate was 6 – 8 drops per minute and fractions were collected every 2 mL in microtubes. Fractionation of the chloroform crude extract was carried out in the same way as the methanol-water crude extract fractionation method with different ratios of the stepwise elution gradient used. The eluent ratio (Hexane: Acetone) used sequentially is 7:3; 3:7; 1:1 and 1:9 (v/v) (Panjaitan, 2014).

Cytotoxic Test on Breast Cancer Cells in Vitro

Culture and sub culture of MCF-7 Breast Cancer Cells

MCF-7 cancer cells will be obtained from the cell culture laboratory of the Faculty of Medicine, Teaching Hospital, Mataram University. Cancer cell culture is carried out using cancer cells that have been frozen at -80°C. Thawing cancer cells is done by warming the cells to a temperature of 37°C then adding new medium. Centrifuge at 1500 xg for 5 minutes. Resuspend again using 1 mL of media. The cells were then transferred into a flask filled with 4 mL of complete media (RPMI 1640 Media, Pen-strep 1% (v/v) and FBS 10% (v/v)). Cells were incubated at 37°C, 5% CO2 for 24 hours or until cells were obtained with 80% confluence. Next, the cells can be directly harvested and tested or sub-cultured. Cell harvesting was carried out by changing the growth medium and washing the cells using 2 mL of PBS (Phosphate Buffer Saline). A total of 2 mL of Trypsin-EDTA 0.05% in PBS and incubated in an incubator for 5 minutes. The cells were observed with a microscope and the cells that had separated from the bottom of the flask were transferred into a 15 mL centrifuge tube containing 2 mL of complete media. Next, centrifugation was carried out at a speed of 1500 xg, 4°C for 5 minutes. Cancer cell sub-culture is carried out by transferring the suspension into two to three flasks containing complete media and growing in an incubator at a temperature of 37°C, 5% CO2. The number of cells was counted using a hemacytometer and counter. Cells were stained first using trypan blue in a ratio of 1:1 until a blue color appeared. The staining results are viewed using a microscope. Dead cells will absorb trypan blue so they will appear blue, while living cells will remain transparent. Next, determine how many cells will be used for the cytotoxic test using seaweed extract using the MTT method.

Cell Viability and Cytotoxicity Test with MTT

Cells that are ready to be harvested and diluted using culture media as needed are transferred to a test plate with 96 wells with 100 µL in each well. Next, the cells were re-incubated in a 5% CO2 incubator at 37°C for 24 hours to recover the cells that had been harvested. Meanwhile, extract sample preparation was carried out by making a stock solution by dissolving a certain amount of dry extract with 5% (v/v) DMSO (in sterile ddH2O) to obtain an extract concentration of 2000 µg/mL. Next, graded dilutions were made from the mother liquor using culture media with varying concentrations between

1000 µg/mL – 15.625 µg/mL. Furthermore, after 24 hours, the culture medium was discarded and replaced with media that had been added with extracts and fractions with various concentrations of 200 µL for each well. The same treatment was also carried out on control cells, media control and positive control Doxorubicin 1 µg/mL. Next, the cells were incubated again in a 5% CO2 incubator at 37°C for 48 hours. After 48 hours of incubation, the medium was replaced with 100 µL of new medium to which MTT had been added and incubated for 4 hours. After 4 hours the condition of the cells was checked to see the formation of formazan crystals using a microscope. If formazan crystals have formed, then 100 µL of DMSO is added to each well to stop the reaction and the absorbance is measured on an ELISA reader with a wavelength of 550 nm.

DNA Isolation of MCF-7 Breast Cancer Cells

DNA isolation of MCF-7 breast cancer cells was carried out using a kit from Geneaid according to the instructions provided. Cells that had been treated with the active fraction which had activity to inhibit DNMT1 were incubated for 48 hours. Cells that had been trypsinized and washed with PBS were centrifuged and the cell pellet was taken, added with 200 µL of GB buffer and transferred into a 1.5 µL microtube and incubated at 60°C for 10 minutes. Add 200µL absolute ethanol and vortex. Place the GD column in the collection tube. Transfer the solution to a GD column and centrifuge at 12,000 xg for 2 minutes and discard the supernatant. Add buffer W1 and centrifuge at 12,000 xg for 60 seconds and discard the supernatant. Add 600 µL wash buffer and centrifuge at 12,000 xg for 60 seconds and discard the supernatant. Transfer the column into a new microtube. Add 100 µL of elution buffer and centrifuge at 12,000 xg for 60 seconds. The DNA obtained was observed using 1% (w/v) agarose electrophoresis.

3. Results and Discussion

3.1 Results

Figure 1. Crude extract, the result of maceration and evaporation of one of the green macroalgae.

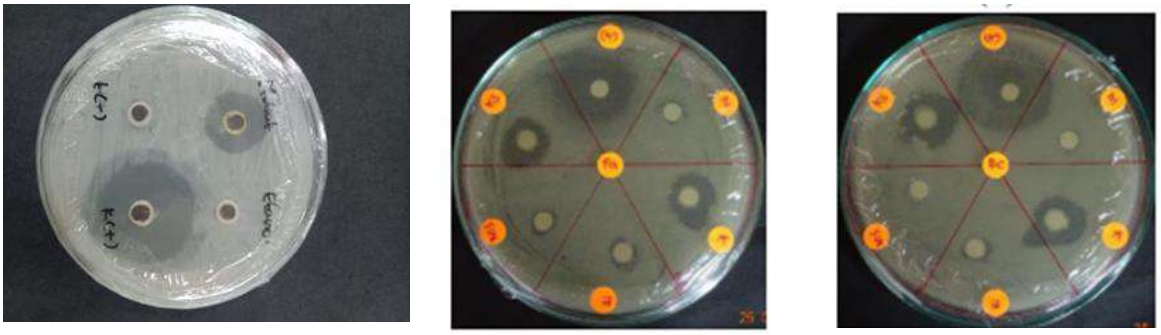

Figure 2. Image of test results for the anti-bacterial activity of several macroalgae.

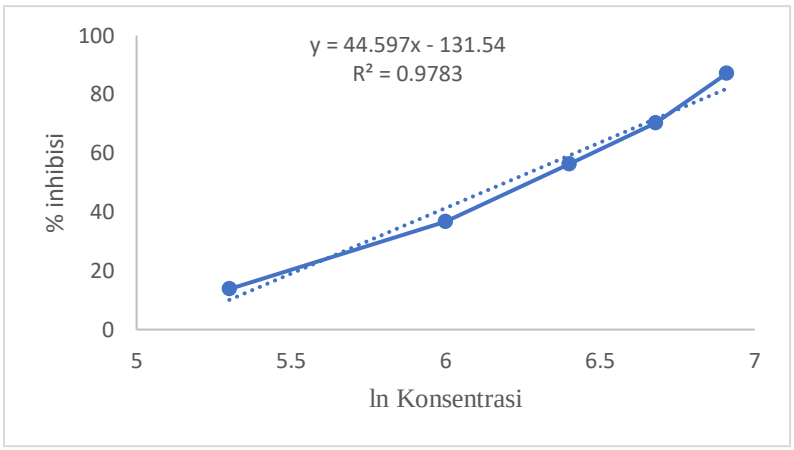

Figure 3. Graph of macroalgae inhibitory test against free radicals.

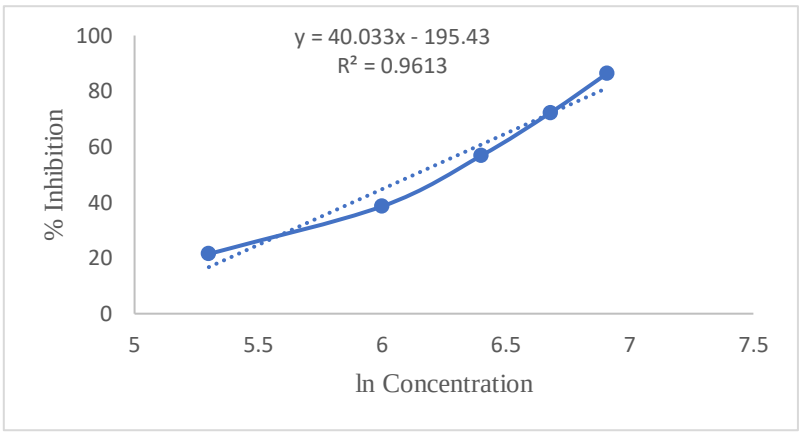

Figure 4. Graph of Vitamin C inhibitory test against free radicals

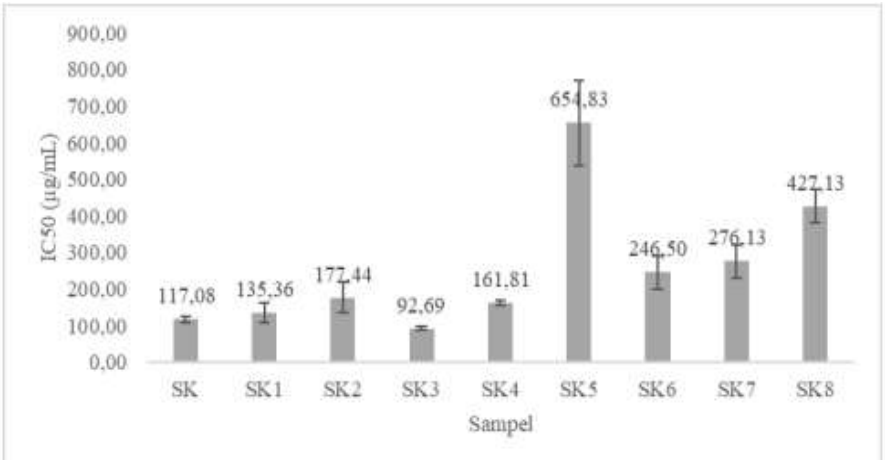

Figure 5. Graph of cytotoxic test results for 9 macroalgae samples

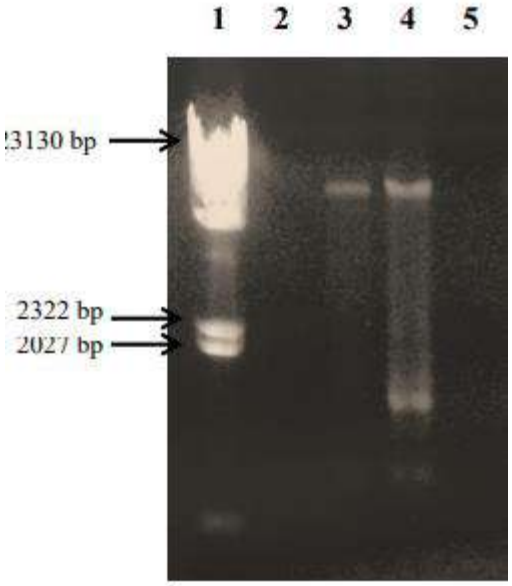

Figure 6. Image of DNA fragmentation results, where number 1 is marker, number 2 is negative control, number 3 is MCF-7 cancer cell DNA, number 4 is positive control, and number 5 is macroalgae sample.

3.2 Discussion

Samples and determinations have been successfully obtained with confirmation of the following brown macroalgae (*Hormophysa cuneiformis, Padina australis, Turbinaria ornata*), green macroalgae (*Ulva intestinalis, Ulva reticulata*) and red macroalgae (*Acanthophora spicifera, Eucheuma sp, Gracilaria foliifera, Wurdemannia miniata*).

The 9 macroalgae were dried first, then the simplicia were made. After that, the compound was isolated using the maceration method using ethanol solvent and evaporation to remove the solvent to obtain a macrolagae extract.

Crude extracts (**Figure 1**) from each (9 types) of macroalgae were also obtained which were then tested for phytochemicals and then tested for antibacterial and antioxidant activity. As for the results, there are those that have antibacterial activity but

no antioxidant activity (weak category antioxidants), conversely there are those that have antioxidant activity but weak category antibacterial activity.

Antibacterial activity test

The antibacterial activity test of macroalgae crude extract was carried out using the Disc Diffusion method and the clear zone formed was observed. Testing was carried out on pathogenic bacteria representing the group of gram-positive bacteria (Staphylococcus aureus and Bacillus cereus) and gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa). The test was carried out by inserting 100 µL of bacterial liquid culture into solid LB media using the spread method. After the bacterial culture had solidified, sterile paper discs were then inserted and 10 µL of sample was dropped onto each paper disc with a sample concentration of 5 mg/mL which was dissolved in each solvent (ethanol) and solvent as a control solvent. The positive control used was Kanamycin with a concentration of 0.5 mg/mL in ethanol solvent. Observations were made by observing the clear zone that formed around the disc paper and then measuring it using a ruler (Esteller and Herman, 2022).

From the results of the antibacterial activity test (**Figure 2**), only the red macroalga *Acanthophora spicifera* had antibacterial activity higher than the positive control (commercial antibiotic in the form of kanamycin) while the other 4 had antibacterial activity but their inhibition zone values were still below the positive control, the remaining 3 did not even have inhibition zone values.

Antioxidant Activity Test

Antioxidant activity was carried out using the DPPH (2,2-diphenyl-1-picryhydrazyl) method, which is a free radical compound. The measurement carried out using this method is the ability of a compound to capture free radicals. DPPH has a maximum absorption at a wavelength of 517 nm (Chakraborty and Dhara, 2020).

The ability of a compound to inhibit the DPPH free radical compound depends on its ability to form pairs with the free electrons of the DPPH compound (O'Sullivan, 2015). The antioxidant activity test using DPPH is an initial analysis used to determine the potential of an extract as an antioxidant. The DPPH test has been widely used to screen an extract that is thought to have bioactivity as an antioxidant at small concentrations. Antioxidant activity is related to the ability of a bioactive compound to donate hydrogen (H) to form reduced DPPH (DPPH-H) (Chakraborty et al, 2017).

The IC_{50} value was obtained (**Figure 3 and 4**) which was the largest and greater than the positive control in the form of Vitamin C in the green macroalga Ulva reticulata of 56.144 in the very strong inhibition category (the IC_{50} value of vitamin C was 57.378 in the strong category).

Cytotoxic Test

The cytotoxic test results showed that the best inhibitor in terms of the IC_{50} value was the green macroalgae Ulva reticulata of 92.69 (**Figure 5**), so the anticancer activity test using the DNMT1 enzyme inhibition method was continued only for this green macroalgae.

DNMT1 activity and DNA fragmentation assay

The results of tests carried out on the active fraction obtained for DNMT1 activity showed that green macroalgae could inhibit DNMT1 activity by 51.09% at a concentration of 163 µg/mL and could inhibit DNMT1 activity by 67.60% at a concentration of 93 µg/mL. These results indicate that green macroalgae have excellent potential to inhibit epigenetic mechanisms through DNMT1 activity. Observations of DNA fragmentation in MCF-7 cancer cells showed that the two active fractions of macroalgae extract were not able to trigger apoptosis in MCF-7 breast cancer cells. So it is necessary to carry out more research on observing DNA fragmentation in MCF-7 cancer cells to produce better data. Results number 4 & 5 (**Figure 6**) should be the same if you want to get fragmentation results that show green macroalgae are capable of becoming natural compounds as anticancer

Conclusion

The research results show that there are macroalgae that have antibacterial activity but do not have antioxidant activity (weak category antioxidants), conversely there are macroalgae that have antioxidant activity but weak category antibacterial activity, and none have anticancer activity. Where the macroalgae with the best antibacterial activity is the red macroalgae *Acanthophora spicifera* and the macroalgae with the best antioxidant activity is the green macroalgae *Ulva reticulata*.

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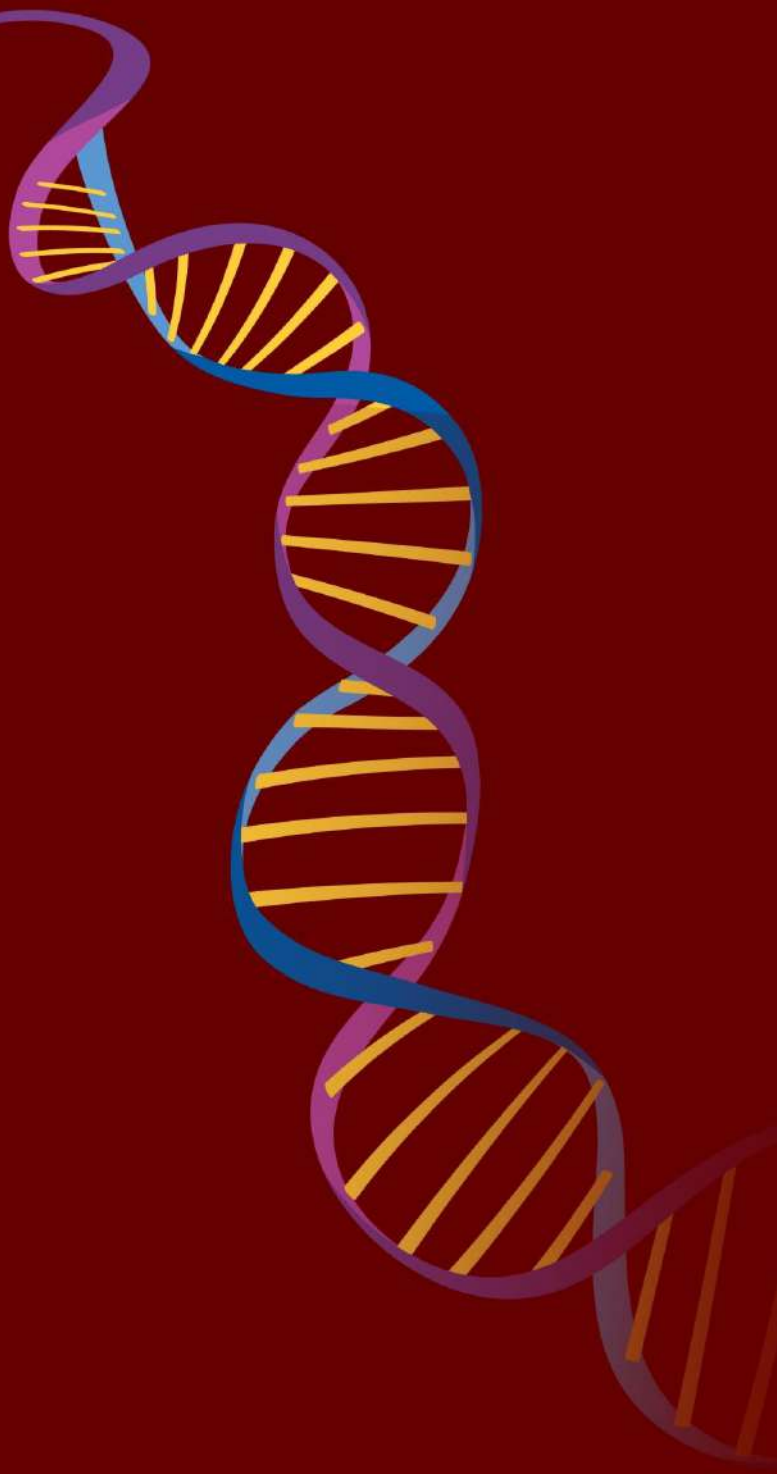

Editorial Office:
Universitas Islam Negeri Mataram
Jalan Gajah Mada Nomor 100 Mataram, 83125
Website: https://biota.ac.id/index.php/jb
email: jurnalbiota@uinmataram.c.id
Telp: (0370) 621298